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COLD SPRING HARBOR
SYMPOSIA ON
QUANTITATIVE BIOLOGY

COLD SPRING HARBOR
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QUANTITATIVE BIOLOGY

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COLD SPRING HARBOR SYMPOSIA ON QUANTITATIVE BIOLOGY



Cold Spring Harbor Symposia on Quantitative Biology are an experiment in scientific procedure, a natural outgrowth of the Biological Laboratory's policy of fostering a closer relationship between biology and the basic sciences.

Each summer* the Laboratory invites a group of mathematicians, physicists, chemists and biologists, actively interested in a specific aspect of quantitative biology, or in methods applicable to it, to take part in conference-symposia which have certain unique characteristics. Among these are the long duration, five weeks; the fact that no time limit is placed on discussions following the presentation of formal papers; the purposefully small size of the attending group at any one meeting in order that discussion may be stimulated; and the fact that participants in a discussion aid in its revision, and thus, in a sense, the resulting volumes give a true picture of the stage of development of the material considered at the time the volumes are prepared. The accuracy of the picture is further insured by including the unpopular side of controversial questions.

Some of the participants are in residence at the Laboratory during the five weeks' period, or even throughout the summer. Others are concerned primarily with a more limited aspect of a given group of symposia and remain in residence for a week or two. Certain participants who cannot be present are invited to transmit discussion by mail. Investigators working upon problems under discussion may attend meetings, programs of which are sent on request.

This year eleven full days and fourteen half days were given to the presentation of papers and subsequent discussion. In addition, many hours were spent in careful consideration and revision of the discussions as originally presented. The remainder of the five weeks was available for informal consultation and conference.

The immediate value of conference-symposia, as conducted at Cold Spring Harbor, is obviously greatest to those taking part in them. At the same time, since large attendance would interfere with certain unique advantages of these symposia, the papers and discussions are being made available with the least possible delay, and at less than cost of publication alone, to scientists at large through the present volume.

* The conference-symposia of 1933 were concerned largely with surface phenomena; those of 1934 with some aspects of growth; those of 1935 with reactions and processes initiated by light. It is likely that nerve, muscle, and other phenomena of irritability will form the basis of the discussion in 1936.

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Introduction to Volume III

If a title were to be chosen for Volume III of Cold Spring Harbor Symposium on Quantitative Biology it might be: The Interaction of Ourselves and Things Around Us with Light.

Viewed in this way the contents have a unity which they certainly have in nature but which they are, perhaps, not permitted to enjoy in the usual university curriculum or scientific meeting. Dividing any part of natural science into component parts is like dividing a region of land into fields. In such divisions of land, it usually happens that fences or stone walls are built. Weeds, shrubs and trees gain a footing in natural succession along the walls and fences: then it becomes difficult to look from one field to the next, and absolutely impossible to see into a field two or three fences away.

Fortunately there is a tendency developing in the natural sciences to cut down the hedgerows at least, and, as fast as possible, to remove the fences. The interaction of light with ourselves and things around us is particularly interesting in this connection because the process of removing the boundary trees has progressed farther here than in many other "fields".

In photosynthesis, for example, one finds a body of knowledge and an experimental technique which could not have been developed as far as they have without appreciable use of both physics and chemistry. Such use has brought a knowledge of the structure of chlorophyll molecules, both *a* and *b*, and of a number of their derivatives; it has given us the current method of testing the purity of extracted chlorophyll, by observation of the absorption spectrum of the extract; it provides the indication that certain parts of a chlorophyll molecule, namely the magnesium and the phytol groups, are not intimately involved in its photodecomposition; upon it is based such information as is at hand concerning the roles of light intensity, of respiration, of temperature and of the enzymic, or Blackman, factor. Recent work on the photosynthesis of bacteria offers findings which make feasible a comparative biochemistry of photosynthesis and a basic mechanism for the oxidation-reduction process involved. Indeed, some of the data which are available are of a nature which make mathematical studies of the kinetics of photosynthesis attractive.

Similarly in other aspects of photobiology there is ample evidence in this volume that up to date chemical and physical methods are being used comparatively extensively and successfully.

Nevertheless, those who look at this time for an unobstructed view into all the fields concerned, will be disappointed. One difficulty will be found to be the fact that present knowledge and theories of photochemistry developed by physicists and chemists are concerned primarily with gases, while we, and many of the things around us, are primarily aqueous systems. Furthermore, by no means all photobiologists and medical men have yet made adequate use of existing chemical and physical knowledge, a fact which is pointed out many times in this volume, and which is apparent in many ways, perhaps particularly in the thus far limited use of monochromatic radiation.

Investigators in the natural sciences will be interested in Volume III because it, like the foregoing volumes, contains the present information and theories of many contributors concerning a sector of modern research.

ABSORPTION SPECTRA AND PHOTOCHEMISTRY, WITH SPECIAL REFERENCE TO WATER SOLUTIONS

GEORGE SHANNON FORBES

Whenever the findings of photochemistry regarding molecular transformations confirm those of spectroscopy, the outcome is reassuring, while any inconsistencies which remain indicate the proper direction for new experiments. The higher oxides of nitrogen, which have been very carefully investigated, serve to illustrate at once the general need for such co-ordination, and the degree of certainty likely to be attained in the interpretation of a fairly simple reaction.

An equilibrium mixture of nitrogen dioxide and tetroxide exposed to violet and ultraviolet light produces nitric oxide and oxygen. Holmes and Daniels¹ calculated ϕ , the quantum yield, i. e.,

$$\phi = \frac{\text{molecules of NO formed,}}{\text{quanta absorbed}}$$

from the rate of increase of the pressure with the energy flux at 436, 405, 366 and 313 $m\mu$. Since pressures were low, the recombination $2\text{NO} + \text{O}_2 \rightarrow 2\text{NO}_2$ was relatively slow, so that the photochemical reaction rate could be calculated with fair accuracy from the pressure change. It proved better to introduce excess of solid nitrogen pentoxide N_2O_5 into the reaction cell; at 0° C its vapor pressure remained constant at 51.5 mm. If $\lambda \geq 300 m\mu$, this vapor (N_2O_5) is transparent and unreactive. The reaction with nitric oxide, $\text{N}_2\text{O}_5 + \text{NO} \rightarrow 3\text{NO}_2$, is so much more rapid than the recombination $2\text{NO} + \text{O}_2 \rightarrow 2\text{NO}_2$ that the latter no longer has to be considered, and ϕ can be measured in terms of decomposition of pentoxide. Such use of an acceptor for reaction products which recombine rapidly may prevent very serious errors in estimation of quantum yields. For instance, irradiated compact silver bromide in air yields less than 0.01 atom of evolved bromine per quantum, but with finely divided material in a nitrite solution, ϕ is practically unity.

Several corrections were required in the nitrogen dioxide photolysis, notably that for absorption of part of the incident light by tetroxide. I have plotted the absorption coefficients of Holmes and Daniels against wavelength (Fig. 1). In view of the banded structure of the gas at the comparatively low pressures (4-30 mm.) which prevailed, such results might have to be used with caution if higher pressures,² or a different light source were to be employed. The total light absorbed is now apportioned between the two with the help of the equilibrium constant, $K = p^2\text{NO}_2/p\text{N}_2\text{O}_4$, and measurements of total pressure.

ABSORPTIONS AND QUANTUM YIELDS

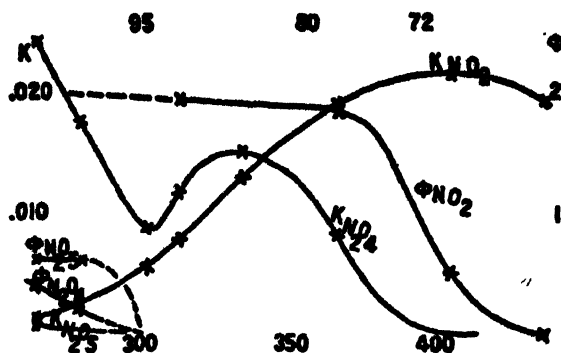


FIGURE 1

To determine the amounts of nitric oxide produced from dioxide and tetroxide, respectively, gross quantum yields for various mixtures were plotted¹ against fraction of light absorbed by NO_2 at 366 $m\mu$ and again at 313 $m\mu$. By extrapolation it appears that ϕ approaches 2 for complete absorption by dioxide. Therefore N_2O_4 , at these wavelengths at least, and presumably over the range 400-300 $m\mu$ acts merely as an inner filter, so that

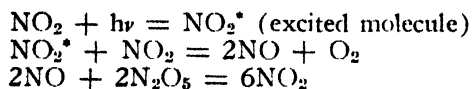
$$\phi = \frac{\text{molecules of NO formed.}}{\text{quanta absorbed by NO}_2}$$

When two or more absorbing substances are present, it is evident that not even a qualitative statement regarding the photochemical reaction is safe without analysis of quantum yields by some procedure equivalent to that of Holmes and Daniels.

Further, it appears that no photochemical reaction should be postulated just because the absorbed quanta exceed the activation energy for the corresponding chemical reaction. Thus, as Norrish pointed out³, the quanta at 436 $m\mu$ (65,000 cal./mol) are nearly twice the activation energy (33,000 cal./mol) of the reaction $\text{NO}_2 = \text{NO} + \text{O}$ and more than twice the heat of reaction (26,000 cal./mol). Nitrogen tetroxide is found to be unreactive throughout the whole of the spectral region involving its first excited electronic state, even at 300 $m\mu$ (94,000 cal./mol) where the energy exceeds by 30,000 cal./mol that at the threshold wavelength (420 $m\mu$ roughly) for the decomposition of the dioxide. Dawsey⁴ examined the spectrum of $\text{N}_2\text{O}_4 \rightleftharpoons 2\text{NO}_2$ and found a continuum increasing from 382 $m\mu$ which

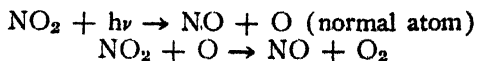
he interpreted in terms of the dissociation reaction $\text{N}_2\text{O}_4 + h\nu \rightarrow 2\text{NO} + \text{O}_2$ (excited molecule), which seemed the more reasonable as even 75,000 cal./mol at 382 $m\mu$ met the energy requirement as figured by him from reaction heats. The results of Holmes and Daniels, however, show conclusively that the tetroxide decomposes only beyond 300 $m\mu$, where a still higher electronic excitation is possible. Holmes and Daniels suggest that the energy up to 300 $m\mu$ is expended in electronic excitation, but still the energy increases by 20,000 cal. in going from 380 to 300 $m\mu$, that is, by an amount one and a half times the thermal heat of dissociation of tetroxide. They also suggest that dissociated tetroxide recombines before photochemical reaction of dioxide can occur. It is unfortunate that increasing dissociation would hinder any test for inception of photochemical activity ($\lambda > 300 m\mu$) at high temperatures where in general many quanta are absorbed by molecules already possessing considerable vibrational energy. Quantum yields in the region $\lambda > 300 m\mu$ would possess independent interest, but would throw little light upon the problem above outlined.

Insecure as well are conclusions regarding primary photochemical acts which are not backed up by spectroscopic evidence. Norrish (1929)³ proposed the following scheme for decomposition of the dioxide:



to which Holmes and Daniels agreed. Now the absorption spectrum of the dioxide at low pressures has been frequently examined. Under high dispersion a series of bands, with fine structure, is observed from 6500 Å. These begin to be diffuse at 4000 Å and become more so up to 3000 Å after which no great change is noted to 2549 Å, when the bands suddenly become sharp again, apparently due to attainment of a new electronic state. According to Henri⁵, Mecke⁶, Franck, Sponer and Teller⁷ this washed-out appearance of the bands from 4000 Å to 2549 Å indicates *predissociation*. As this phenomenon will receive adequate treatment in the papers to follow, it will suffice for the moment to characterize it as a switch from a stable energy level in a molecule to an unstable level of equal energy, so that the molecule flies apart as a consequence. According to Mecke the process in the first banded region is $\text{NO}_2 \rightarrow \text{NO} + \text{O}$ (normal atom), the energy requirement for which is 72,200 cal. per mol¹¹, corresponding to 6×10^{23} \times the energy of a quantum at 3800 Å. From this standpoint 3800 Å appears as the maximum

wavelength at which this reaction would be possible without some source of additional energy. At least when $\lambda \leq 380 m\mu$, the following mechanism is preferable if the transition probabilities of the predissociation reaction are at all favorable.



These reactions run with a total energy decrease of some 50,000 cal., and lead again to a quantum yield of two.

The actual quantum yields for NO_2 were plotted against wavelength by Holmes and Daniels. It appears that $\phi = 0.5$ at 405 $m\mu$ where only 70,000 cal. are available, and that ϕ might be as great as 0.1 even at 420 $m\mu$ (68,000 cal.). There is always the possibility that quantum of themselves just too small may be "pieced out" through recently acquired collisional energy but excited molecules having energy 4 cal. above average would be altogether too few to account for this quantum yield. Possibly the correct value of the energy requirement is less than 72,200. However, Franck, Sponer and Teller⁷ argue that a triangular molecule like NO_2 should require energy in addition to the requirements valid for a diatomic molecule (1) to distort the molecule into a form favorable to dissociation and (2) to impart vibrational and rotational energy to the diatomic molecule produced. One might be tempted to concede that Norrish's excited molecule mechanism predominates when $\lambda > 380 m\mu$, were it not for evidence of predissociation down to 400 $m\mu$. It might go even further, for Franck, Sponer and Teller hold that even after the energy of oscillation has sufficed to bring the nuclei of a triatomic molecule into a position where dissociation is possible, the time intervening may be relatively long, so that in terms of the uncertainty principle the lines would remain quite sharp.⁸

Bonhoeffer and Harteck recall the detection by Norrish of fluorescence in NO_2 at low pressures, —strong at 436 $m\mu$, weak at 405 $m\mu$, and practically absent at 366 $m\mu$. They argue that if at 400 $m\mu$ the number of molecules that actually dissociates equals the number of molecules reverting to the normal state with emission of the absorbed quantum, no broadening of the lines in that region would be observable. This tendency operates in the same direction as the uncertainty principle. Such a picture appears consistent with the quantum yield of Holmes and Daniels, 0.5 at 405 $m\mu$.

It may be that the pendulum of photochemical opinion has swung too far toward atomic mechanisms and too far away from activated molecule mechanisms. The victories of the former over

the latter, in earlier days, through the brilliant work of Franck and his school may in part account for this. Also the possibilities for secondary acts are much more clean cut if one starts out with an atom,¹⁰ and the danger of *ad hoc* interpretations correspondingly less; all these considerations tend to make the atomic mechanism more attractive.

Holmes and Daniels also photolyzed nitrogen dioxide (with pentoxide as acceptor for nitric oxide) in carbon tetrachloride. Quantum yields ran from 0.02 to 0.07 with a tendency to increase with decreasing wavelength, as would be expected if the energy in excess of that required for electronic excitation went into vibrational energy. The whole subject of photochemistry in liquid systems is at present developed in much less detail than in gases, so much that some gas photochemists have questioned the scientific value of such studies. However, practically all the systems of photochemical interest to biologists are in the liquid phase, so that no apology is necessary for introducing the subject here, with an attempt to indicate the direction in which the outlook is most promising.

The transition from gas to liquid can be profitably followed in terms of the changes in absorption spectra. The simplest case is an isolated atom, mercury for instance. Measurements by Wood "show that light close to wavelength 2537 Å is weakened by 50% in passing through one centimeter of mercury vapor, $p = 0.0005$ mm. If 6×10^{20} atoms are present in a light path of unit cross section, one quantum in 10^7 is likely to emerge. This illustrates the tremendous selectivity of quantized absorption. High selectivity in absorption holds also for each of the fine lines in the spectrum of molecules of gases at low pressures, though of course each line represents a different transition probability between states.

As pressures are increased, the resonance line of mercury atom broadens out; also the structure of molecular spectra becomes blurred. Under such circumstances all energy levels of the given molecule are displaced continuously by interaction with the valence electrons of each approaching or receding molecule. Such interactions are small with carbon tetrachloride, for instance, but important with molecules which like water have large dipole moments. If polymers or other definite compounds result, a whole new set of quantized levels are set up which in turn interact with neighboring molecules. In consequence the appearance of the spectrum may be radically altered, and the interpretation of the primary act becomes more difficult, even in the gaseous phase. Reversing this train of thought, it is clear that if any apparent continuum breaks up at low pressure

and high resolution to form sharp fine lines exclusively, neither dissociation nor predissociation is *a priori* to be attributed to photons corresponding to that spectral region.

In the liquid phase the blurring of the spectrum reaches its maximum, but electronic transitions are still in general distinguishable. In carbon tetrachloride solution of halogens, for instance, plots of absorption coefficients against wavelength often resemble in general outline that of the vaporized solute,¹¹ and then it is customary to assume that the primary effect of light absorption is the same in both phases. All too often such a correspondence cannot be established, so that the nature of the primary act remains in doubt. Franck and Rabinowitsch¹⁰ hold that in liquids the probability of reactions proceeding through primary formation of activated molecules must be increased, and rate of formation of free atoms must be lower, than in gases. It is to be noted that the presence of a solvent is apt to promote dissociation because of the decrease in free energy when a single solvated molecule passes into two or more solvated dissociation products. In a few cases, like the uranyl ion¹² or the porphyrins,¹³ the absorbing valence electrons are sheltered, and their transitions are little affected by solvent molecules so that sharp bands are observed in solution, especially at low temperatures. With uranyl and quinine ions in solution, deactivation by collision with solvent molecules is so inefficient that absorbed quanta are re-emitted in part as fluorescent light. The fluorescence of uranyl and of quinine ions can be partly quenched by adding such ions as Cl^- , Br^- , CNS^- and I^- , the effect increasing in the order given.¹⁴ The same ions likewise decrease the efficiency of the uranyl ion as a sensitizer for oxalic acid.

The photolysis of oxalate in the presence of uranyl ion, in water solution, to form carbon monoxide and formic acid is advantageously interpreted in the light of absorption data. When oxalic acid is added to uranyl ion, the absorption coefficient increases¹⁵ as molecules of uranyl oxalate $\text{UO}_2\text{C}_2\text{O}_4$ (known through conductivity measurements¹⁶ to be feebly dissociated) are formed. At the same time the quantum yield for the photolysis increases. Drs. W. G. Leighton¹⁵ and F. P. Brackett, Jr.¹⁷ in my laboratory have calculated from the absorption coefficient of free uranyl ion and of uranyl oxalate the concentrations of these individuals in various solutions. They have apportioned the light between the two, and then assuming that free uranyl ion acts essentially as an inner filter and contributes negligibly toward the total relation they have calculated "net" as contrasted with "gross" quantum yields. Heidt and Daniels further proved¹⁸

that crystallized uranyl oxalate dissolved in water only slightly acidified with oxalic acid gives the same quantum yield as uranyl sulfate plus five or more molecules of oxalic acid,—the amount necessary to bind practically all of the uranyl ion in spite of the tendency of liberated hydrogen ion to reverse the reaction.

It is hard to escape the conclusion that the quanta absorbed by uranyl ion are more frequently effective when transferred across a molecule of uranyl oxalate than when transfer must wait upon collisions. Since the carbon-carbon linkage which is to be ruptured is separated from uranium by oxygen ions, the energy must be propagated through the latter. The propagation of energy through more complicated molecules has been studied intensively in my laboratory. Before examining the photochemical evidence, some consideration of absorption spectra of such molecules is desirable.

Di- or triatomic molecules can be successfully treated as single electronic systems,—indeed almost as if they had coalesced into a single atom—and the interactions (though already quite complicated) can be evaluated accordingly. Such studies indicate what are the possibilities of reaction of each molecule. Large organic molecules can be treated, in the first approximation (to use the language of Norrish¹⁹) as assemblages of covalent groups where each group largely retains its individuality, and tends to contribute its own absorption spectrum to that of the molecule as a whole. Mecke²⁰ has shown, for instance, how small are the changes in nuclear separation and consequently in binding energies when free radicals such as OH, NH, CH, C₂, CN enter into formation of stable compounds. The extent to which the individuality of a constituent group is changed, that is, the relative importance of its interactions with other atoms and groups in the molecule, is also to be inferred from spectral data.

The persistence of the individuality of a characteristic group has been exemplified in an extended research^{21,22,23} by Dr. Heidt and myself upon quinine and ten of its derivatives in water solution. The quinine molecule consists of a quinoline group connected through a secondary hydroxyl group to a ring structure saturated except for a vinyl group marked. Plots of absorption coefficients against wavelengths for quinine and the derivatives²² resemble that of quinoline, from which all are derived.

All these compounds are oxidized by chromic acid in presence of light, the dark reaction being unimportant at the low concentrations prevailing. It has been conclusively proved that the chromic acid acts as an inner light filter exclusively.²⁴

The quantum yield, ϕ_q , is given in terms of light absorbed by quinine or by a quinine derivative. Quinine is stable against chromic acid in blue light, 436 m μ , where the absorption of quinine is negligible, but is decomposed at 405 m μ , where absorption is considerable. If quinine is replaced by cinchonine, the absorption curve of which resembles that of quinine shifted some 2000 cm⁻¹ toward the ultraviolet, no photolysis occurs even at 405 m μ , but at 366 m μ the quantum yield approximates that for quinine at 405 m μ under conditions otherwise comparable. If quinine is replaced by vuzin, the shift is toward the red, and the absorption maximum becomes broader. In consequence of the absorption at λ 436, vuzin reacts with chromic acid in light of this wavelength.

Rabe²⁵ has proved that the secondary hydroxyl of quinine is oxidized in the thermal reaction. This would seem *a priori* probable for the photochemical reaction as well and experimental results point to the same conclusion.²³

It has been shown also²² that ϕ_q has no important connection with the magnitude of the absorption coefficient at a given wavelength. For in the series quinine, optochin eucupin and vuzin, ϕ_q at 405 m μ varies irregularly, and not more than 30% while K changes four-fold. More important it would seem is the fact that this wavelength in all four cases falls upon the same part (here the steep slope) of an absorption curve.

Dr. Heidt and I have suggested²² that the structure of the curves suggests three electronic levels, the vibrational and rotational levels being of course perturbed in all cases to form a continuum. Absorption due to electronic level (1) starts not far from 436 m μ and increases rapidly. That due to electronic level (2) begins at the minimum near 270 m μ and also increases rapidly. That due to electronic level (3) seems to start at about 230 m μ and increases slowly, suggesting that the binding is now looser than in the first two cases, and that the probability of dissociation or rearrangement is greater in this spectral region.

We also excited the fluorescence spectra of quinine and of eight of its derivatives²² with monochromatic light, $\lambda = 366$ m μ . We photographed these on panchromatic plates and analyzed the negatives with a recording densitometer but without discovering any structure. No well defined differences in these fluorescence spectra could be observed, which suggests that the quinoline group is again the dominating factor in the situation. This fluorescence indicates excitation rather than dissociation or rearrangement so long as $\lambda \geq 366$ m μ at least.

Any precise description of the effect of absorption of a photon is impossible, but by way of compensation this question becomes of secondary importance. Apparently energy absorbed by the quinoline group is transferred to the hydroxyl at position (4). If dissociation did occur at this point, collisions with solvent molecules would tend to hold the products *in situ*, as well as any hydrochromate or dichromate ions already nearby. Then one would reckon the outcome not in terms of collision numbers, but in terms of the concentration of photochemical clusters in which the necessary combination of molecules and ions was present, and the relative positions of these more or less favorable to reaction. Such clusters may well approach in behavior that of single big molecules within which activation and dissociation would tend to produce closely similar results, but in which the efficiency of energy transfer from the absorbing quinoline group to the hydroxyl would be the main question.

We do not know the energy of activation for the secondary hydroxyl of quinine and its derivatives, and so cannot state what fraction of the energy of any photon absorbed by the quinoline group must be transferred into this hydroxyl. Some resonance is reasonably inferred. The efficiency of the resonance between the quinoline group and the secondary hydroxyl, where the reaction occurs, seems to be profoundly affected

would suggest interesting generalizations regarding such energy transfers within molecules and clusters.

In making comparisons among quantum yields, one precaution is usually overlooked, the importance of making all comparisons of quantum yield at equal concentrations of activated molecules, which may be taken, in the first centimeter of reaction mixture, for instance, as proportional to E_q , the number of quanta absorbed by quinine in the same zone. Dr. Heidt and I have regulated this concentration both by varying total concentration of alkaloid, and by varying the stop on the front lens of the monochromator. By each method, it is found that ϕ_q falls off at the same rate when E_q increases. This relation is plausibly explained by assuming that the rate of formation of such clusters containing alkaloid, chromic acid and hydrogen ion as are capable of reaction upon absorption of a photon is not rapid in comparison with the rate at which photons are absorbed. As a consequence the concentration of reactive clusters will diminish upon turning on the light, until a photochemically steady state is reached together with a slower rate of photolysis. This is analogous to the electrochemical polarization which occurs when there is a high current density at a cathode immersed in a dilute solution, say, of copper ion. Another way to illustrate the relation is seen in Table I where it is shown that

TABLE I

$[K_2Cr_2O_7]$	$[H_2SO_4]$	[Quinine]	E_o	E_q	ϕ_q
0.00016	0.8	0.000250	6.4×10^{16}	4.9×10^{16}	0.057
0.00016	0.8	0.000025	19.2×10^{16}	4.5×10^{16}	0.059
0.00016	0.8	0.000010	47.0×10^{16}	4.8×10^{16}	0.057

ϕ_q is determined by E_q , not by incident light, E_o .

$\lambda = 366 \text{ m}\mu$, Temperature, 5°

by the mass of the group attached on the opposite side of the quinoline group. For ϕ_q is negligible at $313 \text{ m}\mu$ when hydrogen is found in this position (cinchonine or cinchonidine), 0.013 for hydroxyl (hydrocupreine), 0.070 for hydroquinine (methoxyl), and 0.060 to 0.072 for the derivatives having ethoxyl, isoamoxyl and isoocetoxy, respectively. The wavelength $313 \text{ m}\mu$ used in these experiments falls in each case just to the right of a broad flat maximum of the absorption curve. The greater the mass on the opposite side of the quinoline group, the more likely it is that sufficient energy will pile up in the secondary hydroxyl to bring about oxidation. A heavy group does not act as an energy trap, but rather seems to induce resonance,—possibly by producing some degree of symmetry on opposite sides of the quinoline group. Accumulation of data for an adequate number of related compounds

E_q can be held constant by simultaneous variation of E_o and concentration of alkaloid. The conclusion follows that if a quantum yield falls off with increasing concentration of the photosensitive constituent, then quantum yields at different wavelengths should be compared only for equal concentrations of activated molecules. The importance of this was not realized by us in 1932, and so we were unable to explain the fact that ϕ_q at $\lambda 254 \text{ m}\mu$ came out somewhat smaller than ϕ_q at $\lambda 280 \text{ m}\mu$, though the main trend of the curve was upward. To-day we would be inclined to attribute this to the fact that the K_q is nearly a hundred fold greater at $254 \text{ m}\mu$ than at $\lambda 280 \text{ m}\mu$. At $\lambda 254 \text{ m}\mu$ the reaction must occur mainly in a thin layer next the front window, where the concentration of activated quinine molecules is relatively large. It is quite possible that some other cases of the decrease in ϕ with decreasing

λ (i.e. with increase in the magnitude of the quanta) could be similarly explained.

Dr. Heidt has suggested an additional possibility, not mentioned in papers hitherto published, that a small but important fraction of all the photons are directly absorbed by the secondary hydroxyl of the quinine derivatives containing it. He has expressed an intention to test this possibility, but the substances required to make crucial experiments have not yet been synthesized or else are unobtainable at present. He recalls the observation of Massol and Fauson²⁶ that the absorption threshold shifts from λ 2385 to λ 3430 Å in the series methyl to octyl alcohol, also that the difference in absorption between primary and secondary alcohols is unimportant. To be sure, Massol and Fauson observed these thresholds using a layer of 10 cm. thick, i.e., something like 0.1 mol in a light path of unit cross section, as against 10^{-6} mol , or less, in our experiments. Just what would be the absorption of a secondary hydroxyl having the environment prevailing in the molecule of quinine or a derivative is impossible to predict. The following experiments*, however, are suggestive. The concentration of activated molecules was held substantially constant by the use of stops on the front lens of the monochromator.

λ in $m\mu$	ϕ cinchonine	ϕ quinine ²²
405	no absorption	0.027
366	0.005 ²⁵	0.070
280	0.014*	0.092
313	0.002 ²⁵	0.065
254	0.080*	0.077
208	0.061*	0.105

* hitherto unpublished results by us

It is evident that $\phi_{\text{cinchonine}}$ is small over the spectral region of the first electronic absorption band of quinine which extends from λ 405 to λ 280 $m\mu$, while ϕ_{quinine} is 0.027 even at λ 405 $m\mu$ and has virtually reached its peak at λ 280 $m\mu$. This suggests that quanta absorbed by the quinine group of cinchonine are much less efficiently transferred to its secondary hydroxyl than to the secondary hydroxyl of quinine. The sharp increase in $\phi_{\text{cinchonine}}$ at 254 $m\mu$ therefore tends to support the hypothesis that in this wavelength region the secondary hydroxyl in both alkaloids begins to absorb in lieu of quinine, thus inducing a substantial part at least of the observed photochemical activity of cinchonine, quinine and by analogy the other derivatives also.

The general question of the variation of quantum yield with wavelength has been the theme

of researches by many investigators, which Allmand²⁷ summarized up to 1931. A critical survey of the data reveals the weakness, in many cases, of the experimental methods for determining the absorption coefficients, the energy flux and the amounts of photolytic products and the insecurity of some of the hypotheses suggested by such results. That no simple rule can be laid down is evident as we recapitulate the systems discussed above, and compare them with two others.

Nitrogen tetroxide. No photochemical activity on either side of the absorption maximum at λ 336 $m\mu$, $\phi \text{ N}_2\text{O}_4$ starts from zero and rises slowly when the next higher electronic state is reached.

Quinine. ϕ_q has a maximum at λ 280 $m\mu$, an absorption minimum.

Cinchonine. $\phi_{\text{cinchonine}}$ has a maximum at λ 254, an absorption minimum.

Acetaldehyde.²⁸ A doubling of quantum yield in passing from λ 313 $m\mu$ (which falls upon an absorption band) to λ 334 $m\mu$ which falls directly between two band maxima. Leighton and Blacet think that the underlaid continuum may be responsible for this outcome.

In the cases of cinchonine and acetaldehyde alike, there appears to be some overlapping of absorption bands of active centers.

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DISCUSSION

Dr. Pettit: I would like to ask if it is within the realm of possibility to obtain the spectrum of a single bacterial cell.

Dr. Forbes: I would hardly say that anything of the sort is impossible. Last week, at the laboratory of the Public Health Service at the Harvard Medical School, they showed me an ultraviolet microscope with which they took ten photographs of a single cell at different optical levels. To obtain a complete absorption spectrogram of a single cell would be much more difficult than to suspend many cells at once in a medium having negligible absorption. Perhaps you have in mind the absorption of different parts of the same cell, which would be a still more complicated problem.

Dr. Pettit: What I had in mind was a basis of differentiation between bacteria. For example, if it were possible to get absorption bands characteristic of each type of cell we would then have a single criterion to which reference could be made.

Dr. Forbes: It might prove easier to obtain transmissions at a number of different wavelengths successively, using monochromatic light in each case. There is need for refinement of method, and a great deal of patience.

Dr. Mestre: I would like to amplify Forbes' remarks concerning the difficulties in the way of determining the absorption spectra of bacteria. The extremely small size of most bacteria would seem to exclude from consideration any such direct spectrophotographic method as was used by Becking and Ross with single cells of *Euglena*. The conventional methods of photographic photometry could be applied to a series of photomicrographs made by monochromatic light of various wavelengths, the optical densities of the images of the bacteria being determined by means of a projection densitometer with a small circular aperture instead of the usual slit. While this method does offer the possibility of determining the absorption of single bacteria, it would seem that its most interesting application should be in the study of larger cells where distinct

structures differing in their absorption coefficients are to be found.*

Except in the case of pigmented forms, the absorption coefficients of the substances composing the bacteria are very low at wavelengths outside of the ultraviolet, and there would be considerable difficulty in determining how much of the optical density of the photographic image is related to specific absorption by cell substance and how much to scattering of one type or another. The scattering of light by bacteria is an extremely complex affair which cannot well be discussed here, but it may be pointed out that it is not only a function of the optical system, and of the wavelength used, but also changes rapidly with the physiological state of the bacteria. While the specific absorption is much higher in the ultraviolet, and work in this region offers interesting possibilities, it should be pointed out that much of this absorption is characteristic of proteins in general and will not serve as a criterion for the differentiation of species. Another factor to be considered when working in this region is the possibility of changes in the absorption spectrum resulting from photochemical reactions initiated by the light used for the spectrographic study.

As suggested by Forbes, it is, for most purposes, preferable to work with suspensions of cells. Theoretical considerations and preliminary experiments would seem to indicate that the most satisfactory procedure for studying true absorption due to cell substances is to illuminate the suspension of cells with carefully collimated light and to measure only the deviated transmitted flux, that which is transmitted without deviation being rigidly excluded. Under these conditions, practically all the flux measured will have passed through one or more cells, so that the mean light path through cell substance, and hence the true absorption, will be maximal.

In conclusion, it should be remembered that both optical and chemical studies have shown that the physiological changes which occur during the growth cycle of bacteria are so rapid and so profound that it is to be doubted whether single determinations of the absorption spectrum at an indefinite point in this cycle could ever be used as a general criterion for the identification of species.

Dr. Kistiakowsky: It seems to me that an assumption of free radicals formed upon absorption of light by quinine will account for the

* The photomicrographs of various cells, with ultraviolet light, by Wyckoff (Cold Spring Harbor Symposia on Quantitative Biology, **II**, 1934); and the observations of Swann and del Rosario on *Euglena* seem to indicate that there are many possibilities in this direction.

change of the quantum yield with light intensity equally as well as the explanation advanced by Forbes. One has only, then, to assume that the free radicals recombine to some extent, the more so the higher their concentration. There is a considerable body of evidence showing that most organic molecules give free radicals upon absorption of light of sufficiently short wavelengths and I do not see why quinine and related substances should be exceptions.

Dr. Forbes: Owing to the impact of solvent in a solution, the diffusion of dissociation products away from each other would be retarded. Over the short period intervening between the absorption of a photon and the reaction (or deactivation) of quinine, it would be difficult to distinguish between an activated and a dissociated molecule.

Dr. Kistiakowsky: I doubt whether, when a larger molecule is decomposed into two free radicals in a solution, these will recombine before they have an opportunity to diffuse away from each other. Upon decomposition, free rotation of the radicals will set in and it will then be rather unlikely that the free valences find themselves in the correct orientation for a recombination. Besides, we have ample evidence that free atoms are formed in solution just as easily as in the gas phase. For instance, the oxidation of oxalic acid by iodine proceeds in solution through a very clean-cut atomic mechanism. The atoms formed from a single iodine molecule upon light absorption do separate in the solution, as evidenced by the duration of reaction chains which last a tenth of a second or even longer.

Dr. Forbes: Perhaps a better test of dissociation would be some evidence that reorganization of at least one dissociation product had followed the absorption of a photon. But if the occurrence of free rotation precluded the ready recombination of dissociation products, one might expect that these would react quite completely with hydrochromate ion, in the case considered. The quantum yields actually observed are, however, quite small.

Dr. Noyes: In connection with Kistiakowsky's discussion concerning recombination of the products of photochemical dissociation, account should be taken of the shape of the light beam.

Dr. Forbes: The effect, of changing intensity, upon the quantum yield was small in the quinine-chromic acid reaction and not detectable in the case of uranyl oxalate. In cases where the square root relationship obtains, the distribution of energy in the light beam evidently becomes a matter of great importance.

Dr. Noyes: The distribution of absorbed energy among two or more absorbing molecular species must be made with due care. In a region of banded absorption, pressure effects and specific interactions may be important even though each substance, alone, obeys Beer's law.

Dr. Forbes: A mixture of nitrogen tetroxide and dioxide at variable pressure would be a case in point. On the other hand we have found the absorption coefficients of quinine and chromic acid to be nearly independent of concentration in normal sulfuric acid at constant temperature. That of uranyl oxalate in dilute oxalic acid is unvarying if temperature is held constant.

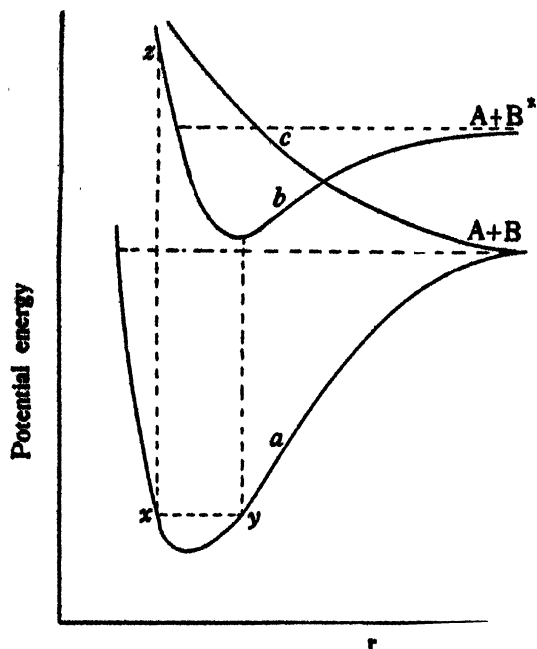
Dr. Noyes: One other point should be emphasized here. Spectrum work on complicated molecules with prism spectrographs of low resolving power often leads one to interpret the spectrum as predissociation when in reality it is not. Most molecules with atoms even as heavy as oxygen and nitrogen require very high resolving power. In most cases, the resolving power is limited.

Dr. Forbes: A grating of sufficiently long focus and resolving power should show definitely whether an apparent continuum is actually continuous or discrete in structure.

Dr. Noyes: More twenty-foot gratings should be used in chemistry departments.

Dr. Rollefson: I would like to suggest a possible explanation for the change of quantum yield with wavelength. Let us consider that the excited molecule produced by the action of light may return to the normal state with emission of a quantum of light or the molecule may go over into another state which reacts either directly or via a dissociation process. If we represent the different electronic states by the potential energy curves *a*, *b*, and *c* in the figure then we shall assume that the absorption of light transfers the molecule from the state represented by *a* to the state represented by *b*. If we reach a point on *b* which corresponds to an energy equal to or greater than that at the intersection of *b* and *c* then we must consider the possibility of a change from *b* to *c*. Even if the energy supplied by the absorption of light is not sufficient to bring the molecule up to the energy corresponding to the intersection of *b* and *c* there is a possibility of acquiring the small additional amount necessary by what Forbes has called a piecing-out process. Now if we consider excitation by the absorption of light of various wavelengths, to different vibrational states of molecules having the electronic configuration corresponding to *b*, then the number of excited molecules which go over to *c* will depend upon how near the molecule comes to the condition represented by the intersection of *b* and *c* and how long it remains in that condition. (If

ABSORPTION SPECTRA IN PHOTOCHEMISTRY



we apply the Heisenberg uncertainty principle to the system we must realize that the intersection which appears as a point in the diagram is really spread over an area so that we have a gradual change in the probability of transition rather than an abrupt one.) Therefore as the amount of vibrational energy in the state *b* increases, the probability of a transfer to *c* will increase at first and thus the number of excited molecules which react will increase. After the amount of vibrational energy has become so great that the system passes the intersection of *b* and *c* rapidly, then the system will not remain in the vicinity of this intersection long enough for the transfer to occur and therefore at the wavelengths corresponding to such states there will be fewer molecules reacting. In terms of quantum yields we should find in the most general case that the quantum yield would increase with decreasing wavelength and then decrease. In particular reactions only part of this variation may be observed, as the molecules involved may absorb only those wavelengths which transfer the molecule to states represented by *b* on one side or the other of the intersection with *c*.

These views can be tested by making accurate measurements of the quantum yield over a wide range of wavelengths, which is often very difficult from an experimental standpoint.

Dr. Forbes: Gas reactions yield the most clean-cut interpretations owing to the greater simplicity of such systems.

Dr. Noyes: I would like to know whether the spectrum of nitrogen tetroxide is continuous.

Dr. Forbes: Harris (Proc. Nat. Acad. Sci. 14, 690 (1928)) states that its spectrum is continuous in the gas phase. Two bands, one with a minimum at 350 $m\mu$ and another with a maximum of still shorter wavelength merge at high pressures into one continuous band extending from 400 $m\mu$ into the far ultraviolet.

Dr. Noyes: Is it possible to explain part of the results of Holmes and Daniels by assuming that in one region nitrogen tetroxide dissociates into two normal dioxide molecules, and in the other region into one normal and one excited molecule, the latter being identical with that produced when nitrogen dioxide absorbs light directly?

Dr. Forbes: That is a plausible interpretation of the process. In the second region the wavelength is less than 300 $m\mu$. The energy, 95,000 calories or more, exceeds by at least 10,000 calories the sum of the heat of dissociation of nitrogen tetroxide into two molecules of dioxide, and the energy of electronic excitation of one of the latter.

Dr. Kassel: In the mechanism suggested by Rollefson to account for a change of quantum yield with wavelength, additional complications must be considered. There has to be some deactivating process, otherwise any molecule reaching state *b* with enough energy will eventually react. If this is a collisional process, the quantum yield will have a pressure dependence. If it is by fluorescence, the emission probability as well as the reaction probability will depend on the energy in the state *b*, and the quantum yield will not be directly proportional to the reaction probability alone. These complications, of course, do not prevent Rollefson's mechanism from operating.

Dr. Bates: There is one other difficulty which occurs to me. Norrish found fluorescence of NO_2 at low pressures only, at high pressure, immediate deactivation on collision with another molecule of nitrogen dioxide. The energy thus transferred is sufficient to bring about thermal decomposition of the molecule.

Dr. Noyes: Is it true that the quantum yield of methyl iodide decomposition is lower in the region of continuous absorption than in the region of banded absorption?

Dr. Bates: In the continuous region, as soon as a few methyl iodide molecules are decomposed, further methyl groups, formed on photodissociation, tend to react with iodine to reform methyl iodide. In the banded region we might have immediate bimolecular reaction without the intervention of methyl groups, or methyl groups may be formed of sufficiently high energy to react with methyl iodide on collision.

THE QUANTUM THEORY OF ACTIVATION AND ABSOLUTE REACTION RATES OF PHOTOCHEMICAL PROCESSES

HENRY EYRING

The absorption of a quantum of light of sufficiently high frequency to cause the dissociation of some molecule into atoms or radicals frequently brings in its train a complicated series of secondary processes. The recombination of the dissociated atoms may lead to a vanishingly small value for the ratio of molecules reacting to light quanta absorbed provided the association process is rapid compared to the alternative secondary processes. If the converse holds for the relative rates we may have a quantum efficiency as high as 10^6 as exemplified by the famous $H_2 + Cl_2 \rightarrow 2HCl$ reaction¹.

The Primary Process. The absorption of light occurs because the atoms being composed of positive and negative charges are pulled apart by the impinging electromagnetic wave associated with a photon of light. Thus if in each of the three directions the electrical field strength of the light wave may be represented by the expression

$$\sum_v E_v \cos 2\pi (\nu t - a\nu) \quad (1)$$

a straight forward analysis² leads to the expression

$$(|ex|^2_{nm} + |ey|^2_{nm} + |ez|^2_{nm}) \sum_v E_v^2, \quad h^{-2} [\sin^2 \pi (\nu - \nu_{nm})t] / (\nu - \nu_{nm})^2 \quad (2)$$

for the probability that in the time t a quantum of light will be absorbed by a molecule or atom having effective values for the square of the dipole moment given by $|ex|^2_{nm}$, $|ey|^2_{nm}$ and $|ez|^2_{nm}$ and a frequency ν_{nm} associated with this transition between the states n and m . E_v^2 is the intensity of the light of frequency ν . From relation (2) we see that the absorption is great if the impressed frequency ν is near the absorbed frequency ν_{nm} ; also that adsorption bands have a finite width which becomes narrower the longer the time t during which the impressed wave train acts on the system. The summation of this probability of absorption over frequency reduces to an integration in which we may replace E_v^2 by $(8\pi/3) \rho_{nm} d\nu$ where ρ_{nm} is the density of photons per unit of frequency in the neighborhood of the frequency ν_{nm} . We thus get for the probability of absorption the expression

$$8\pi^2/(3h^2) (|ex|^2_{nm} + |ey|^2_{nm} + |ez|^2_{nm}) \rho_{nm} t \quad (3)$$

The selection rules for absorption are thus seen to depend on the magnitude of the values of the dipole moments averaged between the two states

which for atoms are in general zero unless the states n and m have their 1 (azimuthal quantum numbers) differing by ± 1 and their magnetic quantum numbers differing by ± 1 or 0. An equivalent statement is that in the collisions of an atom with a photon the electron can change its total angular momentum by ± 1 unit and the z component by ± 1 or 0 units of angular momentum. For molecules we have additional selection rules which are of great importance in determining molecular structure. We shall not consider them further here. The photon excites quadrupole as well as dipole oscillations (although much less frequently) and in this case there may be changes of as much as 2 units in the electronic angular momentum. The linear momentum of a photon is given by the quantity $h\nu/c$ where h , ν and c , are the Planck constant, the frequency and the velocity of light, respectively. Multiplying this linear momentum by the distance r from the photon to the center of the atom we get $(h\nu/c)r$ for the change in angular momentum due to the absorption of a photon. In order for this to be equal to the quantity of angular momentum $h/2\pi$ (1 Bohr unit) lost by the electron we must have

$$2\pi r = c/\nu = \lambda \quad (4)$$

Thus we should think of the photon as being captured in an orbit of such a diameter that the distance around this orbit is equal to 1 wavelength λ just as for an electron in its lowest state we have the distance around the orbit equalling the de-Broglie wavelength.

A molecule or atom which has become excited may lose its energy by fluorescing, or because of its new valence properties it may react, or it may transfer all or a part of its energy by a collision to a second molecule.

As was shown first by Einstein the probability of a system spontaneously radiating is

$$8\pi h \nu_{nm}^3 / c^3 \rho_{nm} \quad (5)$$

times the probability of absorbing³. Here c is the velocity of light and the other quantities are universal constants or have been defined. Combining this factor with relation (3) we find for the probability of a spontaneous transition in the time t the expression:

$$64 \pi^4 \nu_{nm}^3 t / (3c^3 h) (|ex|^2_{nm} + |ey|^2_{nm} + |ez|^2_{nm}). \quad (6)$$

If we set this probability equal to 1 and set the corresponding time $t = \tau$ we obtain, to this approximation, for the mean life of a system

$$\tau = 3c^3 h / [64 \pi^4 \nu_{nm}^3 (|ex|^2_{nm} + |ey|^2_{nm} + |ez|^2_{nm})]. \quad (7)$$

Now if we take x , y and z as approximately 10^{-8} and $\nu_{nm} = 10^{15}$, the approximate frequency for an electron in an orbit, we find $\tau \approx 10^{-9}$ to 10^{-8} sec. τ will be larger for smaller values of the effective dipole moments. For vibrations we have frequencies of the order of 10^{13} so that here $\tau \approx 10^{-6}$ and for rotational transitions we have lower frequencies and correspondingly longer mean lives.

Now the number of collisions which an atom or molecule experiences is proportional to the pressure. At atmospheric pressure a molecule experiences about 10^{11} collisions per second so that if it is electronically excited it will only have about 100 to 1000 collisions (at most) before re-emitting the absorbed light quanta. If a reaction involves an activation energy of E calories the chance of reaction on a single collision is given approximately by the Boltzmann factor $e^{-E/RT}$. If this is to be greater than $1/1000$ at room temperature E must not appreciably exceed 4 kg. cal. Thus we will not observe excited atoms taking part in processes which have any considerable temperature dependence. In condensed phases activation energies as high as 8 kg. cal. may occasionally be observed because of the more frequent collisions.

Probability of Electronic Transitions by Collision. For collisions where the probability of transition from one electronic state to another is low the considerations of Kallman and London⁴ lead to the simplified equation

$$(ih/2\pi) (\delta C/\delta t) = M \exp(-2\pi i E/h) t \quad (8)$$

for the transition probability C as a function of the time t . Wigner⁵ used this equation in an interesting calculation of the rate of the para-ortho conversion of H_2 by paramagnetic molecules. In (8) E is the difference in energy between the initial and final electronic states for the system, i.e., it is the amount of energy which must be supplied from the translation of the colliding molecules; M is the energy of interaction of a kind tending to bring about transition. The actual process considered is the turning around of one of the magnets associated with the nuclear spins of the two hydrogen atoms from a position in which they are anti-parallel to the parallel position. An approaching paramagnetic molecule being nearer one hydrogen nucleus than the other exerts a greater force on the near nuclear magnet thus tending to reorient the pair. M is the magnitude of this reorienting potential averaged between the two states. If the transition takes place the energy of the hydrogen molecule

must jump from an even to the next odd rotational state, which gives the energy E . Wigner, after relating in a reasonable way the duration of the collision time t to the relative velocity and nearness of approach of the molecules, was led to the expression

$$8\pi^2 \mu_a^2 \mu_p^2 r^2 (3h^2 a_s^6 V^2)^{-1} \quad (9)$$

for the probability of transition in a single collision. Here μ_a and μ_p are the magnetic moments associated with the proton nuclear spin and with the molecule colliding with H_2 respectively; r and a_s are the distance between H atoms, and between the hydrogen molecule and the colliding magnetic dipole. The relative velocity V in (9) of the colliding molecules may be related to the reduced mass m and to the absolute temperature T by the equation $mV^2 = 3 kT$. Substituting for V^2 in (9) we obtain

$$8\pi^2 \mu_a^2 \mu_p^2 r^2 m (9h^2 a_s^6 k T)^{-1} \quad (10)$$

Wigner then finds that if a_s is taken as 1 to 2\AA , he gets 10^{-11} to 10^{-13} for the probability of transition per collision in agreement with experiment.

Now we may use (8), (9) and (10) to discuss qualitatively the factors which influence the uncoupling of any two magnetic vectors in one atom or molecule by collision with a second molecule. It should be borne in mind that these relations only apply strictly when the transition probability is low. In the $6^3P_1 \rightarrow 6^3P_0$ transition of Hg we have the electronic spins of the two outer electrons always remaining parallel to each other to give a net resultant magnetic vector of two Bohr magnetons. In the initial state, however, this magnetic vector may be thought of as making an angle of 60° with the magnetic vector arising from the motion of the p electron in its orbit. In the final state these two magnetic vectors have become anti-parallel. Experiment shows that NO and O_2 being paramagnetic are particularly efficient⁶, as we would expect, in quenching the Hg resonance radiation. The molecules are found to have effective radii for this process of 35.3 and 19.9\AA , respectively. In the quenching of the Hg radiation we have (instead of the small magnetic moment μ_p of a hydrogen nucleus) a moment which is larger than the nuclear one in the ratio of the mass of a proton to the mass of an electron. Also because the magnetic moments in the Hg are associated with the electrons in the outer shell instead of with the nucleus they come much closer to an approaching molecule. Both these effects tend to increase greatly the comparative efficiency of quenching processes of the Hg-type over that for para-ortho conversion.

This nearness to the atom surface of the important magnetic moments in Hg makes appreciable interaction possible even with molecules whose magnetic fields fall off much faster than does a dipole moment. Thus molecules like N_2 , CO_2 , CH_4 , H_2O , etc., which are ineffective in the para-ortho conversion are important in quenching. If part of the energy given up by the Hg in its transition is taken up by the colliding molecule as internal excitation the energy E in (8) which measures the residual energy to be taken up from relative translation becomes smaller and the probability of transition correspondingly larger. This, of course, only holds if the interaction energy M (of the Hg magnetic moments with this internal degree of freedom) does not decrease in such a way as to compensate the effect coming from the decrease in E . The experimental aspect of this question is discussed at length by Mitchell and Zemansky.^{6b}

By examining (9) and (10) further we see that the violence of collision has two contrary effects. Increasing the velocity or the temperature T lessens quenching efficiency. On the other hand if the collisions become sufficiently violent a_z will be appreciably decreased, improving the quenching efficiency. At sufficiently low velocities a third effect enters which, due to the approximations in the derivation, does not appear explicitly in (9) and (10); namely if the relative translational energy is insufficient to provide the necessary excitation energy no transition is possible.

Now suppose we are concerned with a transfer of excitation energy between atoms in which the principal quantum numbers change, i.e., the average distance between the electrons and nuclei change, one increasing, the other decreasing. For this case we think of the electron and positive nucleus of one atom as constituting an electrical dipole which interacts with the dipole for the other atom to give the interaction M in (8) while E the energy supplied to (or from) relative translation measures the difference between the two atomic excitation energies. The general considerations then parallel exactly those for magnetic dipoles and we can again use relations (8), (9) and (10). (See the paper by Kallmann and London^{4a} for a discussion of this case.)

Reactions involving a Change in Multiplicity
We take for definiteness the dissociation $N-O \rightarrow N_2 + O$. If we plot the distance between the N_2 molecule and the O as abscissa with energy as ordinate we get Fig. 1. The broken lines are obtained when magnetic interactions are considered, while the continuous lines are obtained when this interaction is neglected.

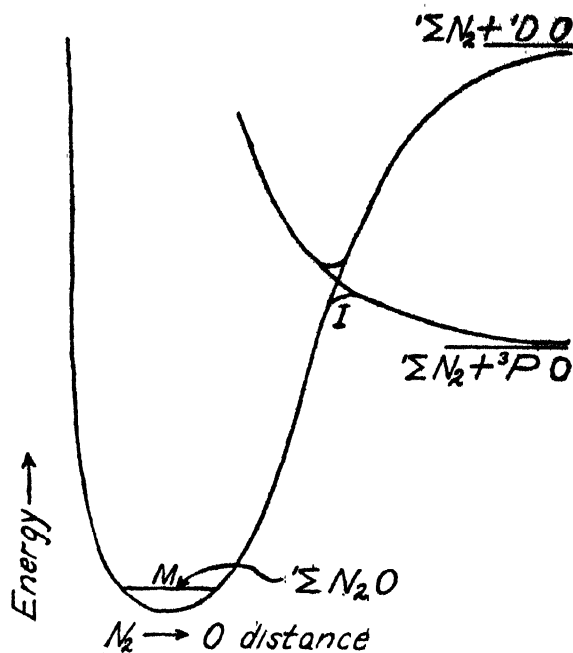


Fig. I Decomposition of N_2O

Note: The curve from ΣN_2O to $\Sigma N_2 + {}^1O_0$ is curve 1 of the text. Curve 2 crosses curve 1 at I. The two short lines, joining respectively the upper and the lower segments of 1 and 2, are referred to as broken lines.

The passage of the point representing the system from curve 1 to 2 involves a change in multiplicity. Now the chance that the system approaching the intersection I from the minimum M shall change over to 2 is given according to Zener⁷ (see also Landau⁸) by

$$P_{12} = 1 - \exp [-4\pi^2 E^2 (hV(s_1 - s_2))^{-1}] \quad (11)$$

where V is the velocity with which the system passes through I. s_1 and s_2 are the slopes of the potential curves 1 and 2 respectively at I while M is the magnetic interaction averaged between states 1 and 2. Experimentally we know the average value of P_{12} (which we write \bar{P}_{12}) for N_2O is about 1/1000 as the reaction proceeds at a unimolecular reaction rate about this fraction of the normal rate. The equation for the specific rate of reaction is given below.

The Absolute Rate of a Chemical Reaction
A chemical reaction involves transfer of atoms from one set of partners to another and might conceivably take place by pulling all the atoms apart and then reassembling them. This, however, would involve an unnaturally extravagant use of energy and does not ordinarily occur except for the very simplest reactions. Generally

in a reaction one or more molecules possessing energy considerably above the average associate to form what has been frequently called an activated complex which then flies to pieces. The number of these activated complexes present at any time multiplied by the reciprocal of their mean life gives the rate of the reaction⁹. Consider the very general reaction:



where n gives the number of molecules of the type A which come together to form the activated complex C and where m gives the number of product molecules of the kind B . For the concentration of C we then have

$$(C) = (A_1)^{n_1} \dots (A_i)^{n_i} K$$

where K is an equilibrium constant. Now the mean life of C is $h/kT\kappa$ where κ is the chance of reaction per single passage of the system from the initial state through the activated state and h/kT is the mean life of a system in any particular translational cell in phase space. Thus we can write for the rate of reaction

$$-d(C)/dt = K\kappa (kT/h) (A_1)^{n_1} \dots (A_i)^{n_i} \quad (12)$$

which gives for the specific reaction rate

$$k^1 = K\kappa (kT/h) \quad (13)$$

For the N_2O reaction we make the identification $\kappa = \bar{P}_{12}$.

The problem in reaction rates then is to calculate the equilibrium constant K and the transmission coefficient κ . The activated complex is like an ordinary molecule in all of its degrees of freedom except the one in which it is flying to

pieces. The activated state corresponds to the particular atomic configuration intermediate between reactants and products, and possesses the indispensable minimum of potential energy necessary for the reaction (unless there is quantum mechanical leakage through the barrier). The activated state is thus the low place in the energy ridge separating the two low regions corresponding to reactants and products. In Fig. I it is represented by the point I; in Fig. II and III, by the line marked reaction shell; in Fig. IV, by the two equally low points in the two passes just preceding and following the symmetrical line which bisects the southwest corner; and in Fig. V the activated state is the point A. Much has been written about the construction of such energy surfaces.¹⁰ Approximate methods of construction are available which answer many of the questions of chemical kinetics. The construction of the accurate surfaces which would suffice to answer all such questions is being approached as time goes on.

Such a surface yields the atomic distances in the activated complex and the vibration frequencies. With these quantities and the same ones for the reactants the equilibrium can be calculated by the well known methods applicable to any equilibrium. Thus for the unimolecular decomposition of the linear molecule N_2O we have for the specific reaction rate constant the expression:

$$k^1 = \bar{P}_{12} I^* I^1 \prod_{i=1}^3 [1 - \exp(-h\nu_i^*/kT)]^{-1} \prod_{j=1}^4 (1 - \exp(-h\nu_j/kT)) [(kT/h) \exp(-E/kT)] \quad (14)$$

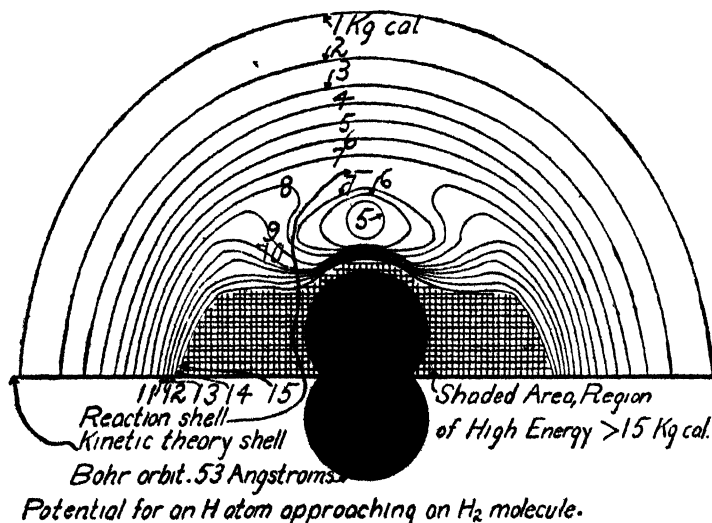


FIGURE II

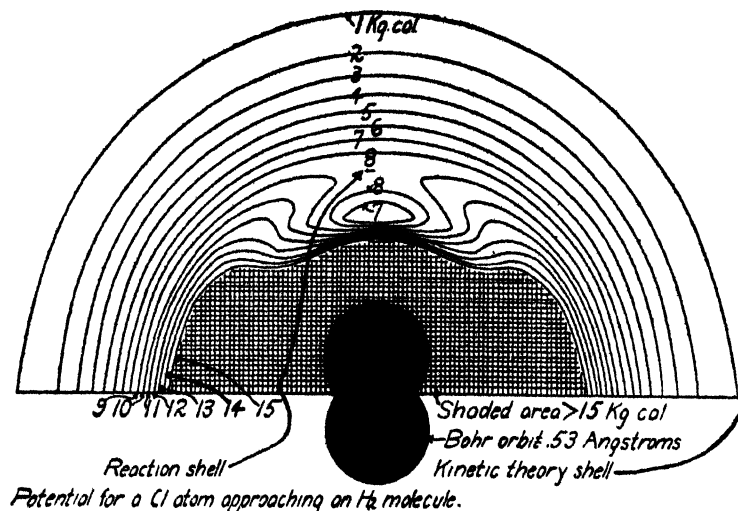


FIGURE III

Here I is the moment of inertia and the ν_j 's are the four vibration frequencies of the normal molecule. The starred quantities have the same significance for the activated complex. The other symbols have been defined or have their usual meaning. In place of the four frequencies of normal N_2O we have three vibrations and a trans-

lation across the activation energy barrier appearing in the activated complex. The translational factor is included in the term kT/h . For any reaction mechanism the equation corresponding to (14) can be written down immediately. We shall now briefly discuss some typical potential surfaces.

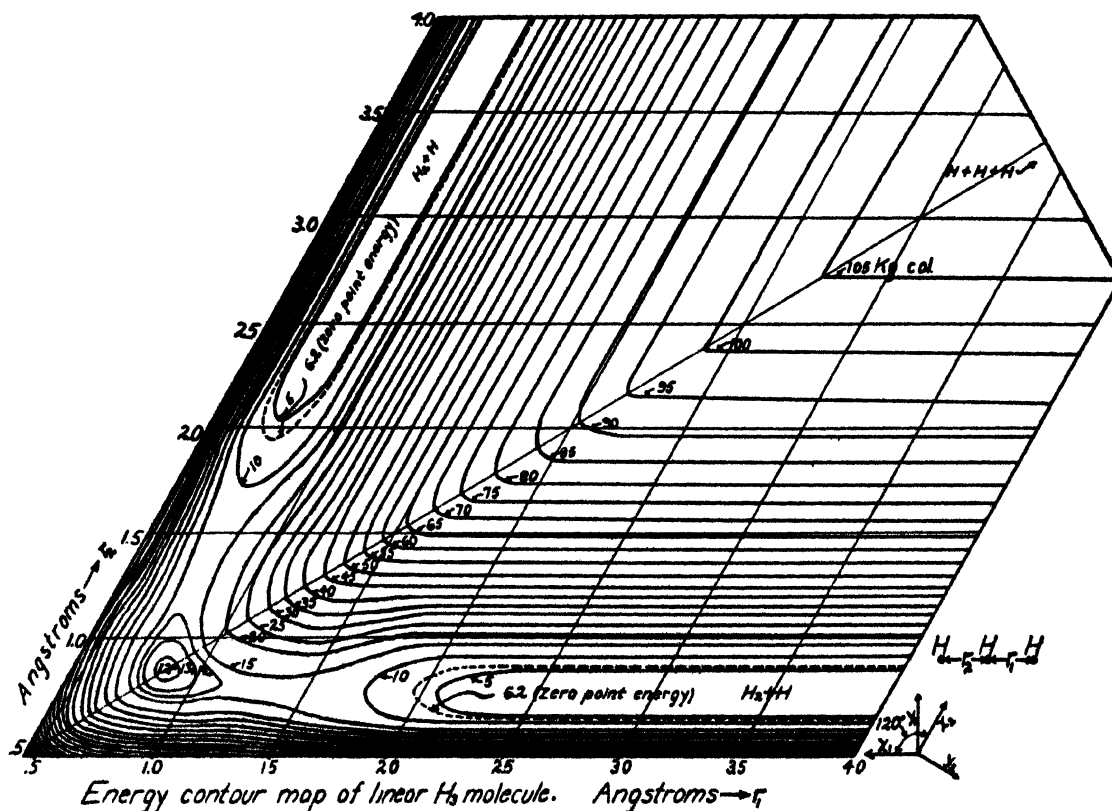


FIGURE IV

For permission to use Figs. II and III the author is indebted to Mr. Joseph Hirschfelder. II shows how the potential energy changes as a hydrogen atom approaches a hydrogen molecule. The energy in kg. calories is indicated by the numbers. The scale of distance is given by the fact that each of the two black circles has a radius of 0.53 Å. If the collision is one with a relative velocity corresponding to less than 7 kg. cal. the H_2 molecule behaves almost as a moderately rigid sphere. Slightly more violent end-on collisions lead to a change in partner for the atoms (a chemical reaction), while collisions in other directions fail unless they are much more violent. Fig. III is a similar diagram for the reaction of a Cl atom with H_2 .

Fig. IV is another aspect of the process pictured in Fig. II. Here energy contour lines are drawn for three hydrogen atoms in a line as a function of the two distances of the outer atoms to the central one. By plotting the two distances on coordinates making 60° with each other the mechanical behaviour of such a linear molecule is paralleled by the motion of a ball on this potential surface. The reaction $H_2 + H \rightarrow H + H_2$ corresponds to a ball moving west up the lower valley crossing through the pass (which is highest just before and just after the symmetrical line) into the second valley. Pelzer and Wigner¹¹ were the first to calculate the absolute rate of a reaction and they used the surface similar to Fig. IV constructed by Eyring and Polanyi.^{10b} They obtained very good agreement with experiment.

Another reaction which also requires this surface for its calculation is the formation of a hydrogen molecule by the collision of three hydrogen atoms. This is discussed in detail in a paper by the present author with Drs. Sun and Gershinowitz¹² to whom we are indebted also for Figs. IV and V. They also obtain good agreement with experiment.

The process in this case corresponds to a ball leaving the plateau marked $H + H + H$ and ending in one of the two valleys, with enough of the energy transformed into motion along the valley so that it can no longer return to the plateau. This transformation occurs negligibly often unless the ball crosses the symmetrical line separating the two valleys. The calculation of the rate then consists in counting the equilibrium number of points, representing the system, which cross this line per second and which in addition fulfil certain conditions on their velocities. These conditions are chosen to exclude systems not beginning on the plateau and ending in the valleys.

Now not only the above linear collisions (and nearly linear ones) may result in molecular asso-

ciation but also those in which one atom is directed toward the center of gravity of a pair colliding along a path normal to their direction of collision. The potential energy for all such configurations of three H atoms forming an isosceles triangle is given in Fig. V. Only half the surface is drawn since it is symmetrical about

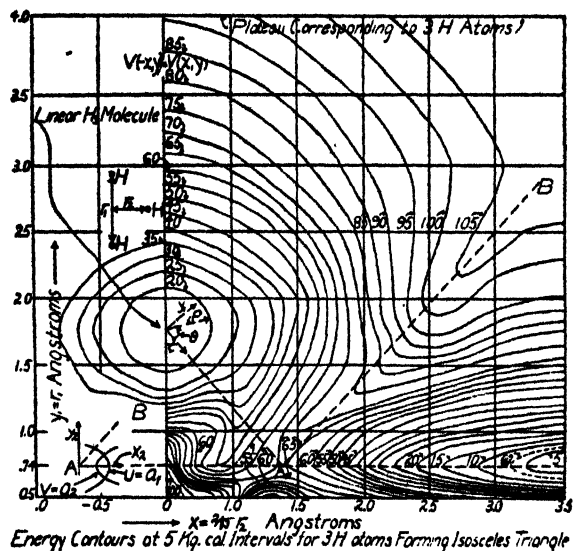


FIGURE V

the line $X = O$. Here as in the linear case if we are given the surface the problem of reaction rates is simply an exercise in statistical mechanics. Thus we must estimate the rate with which particles representing the system pass from the plateau into the east-west valleys at the bottom of the map. A person interested in more details should consult the paper by Pelzer and Wigner^{11a} and by Wigner^{11b}, a series of papers by the present author and his collaborators,^{9,13} and one by Evans and Polanyi.¹⁴ These publications treat the general theory of absolute rates and make many new applications.

In the brief time available it has not been possible to do more than indicate the methods of calculating reaction rates in photochemical systems. It should be clear, however, that theoretical methods are already available for considering most of the important mechanisms and that in certain directions these methods are giving not only qualitatively, but quantitatively correct results.

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DISCUSSION

Dr. Kassel: You made a remark about the two nitric oxide energy curves which I don't quite understand; something about their failure to cross when treated exactly.

Dr. Eyring: If only electrostatic interactions are considered in the Schrödinger equation the energy levels for the triplet and singlet curves cross as indicated by the full lines in Fig. 1. If the magnetic forces between the spinning electrons are also considered we get, instead of the crossing energy levels, the modified curves indicated by the broken lines.

Dr. Kassel: I understand how that works out in other cases; but it seems strange here that in only one crossing out of a thousand the molecule stays in the curve, and in all the others it jumps the track.

Dr. Eyring: How often it jumps the track of course depends on the velocity. For zero velocity the broken curves represent the exact solution of the Schrödinger equation and there is no tendency to jump from one energy level to the other. However, a finite velocity introduces in effect into the Schrödinger equation a perturbation which grows with the time and the velocity; so that the energy levels, or tracks, for the stationary system are no longer quite the proper tracks. The fact that we have jumps or transitions at all arises from the fact that we persist in describing our system in

terms of energy levels which are only approximately correct.

Dr. Rollefson: There is one point which I would like to illustrate with the diagram for N_2O which shows the two electronic states, one dissociating into N_2 and a normal oxygen atom, the other into N_2 and oxygen in the 1D state. In the diagram the latter state corresponds to a stable molecule, whereas the former does not. Now suppose we are trying to decide what will happen if we bring an oxygen atom up to a nitrogen molecule. If we are dealing with a normal atom and molecule, these calculations would lead to the conclusion that a stable molecule would not form, but in this case we can find other states which will lead to the stable molecule which we know exists. In most reactions, however, we are interested in trying to decide whether or not certain intermediates can be formed and we do not know the answer. Is there anything we can do with such a problem except calculate all possible interactions between all combinations of reasonable initial states and see if any lead to the formation of stable molecules?

Dr. Eyring: The only way of arriving at energy levels and eigenfunctions for molecules as complicated as those for N_2O is by use of the laborious variation method or the perturbation theory. The quantum numbers for a central field which apply well to the free O atom must describe very badly the "oxygen electrons" in N_2O . When the levels are far apart, as they are for a hydrogen atom, they still remain quite well separated in a molecule formed from the hydrogen. In most cases, however, we have molecular levels taking on the properties of a number of atomic levels in proportions only determinable by laborious calculations, as you indicate.

Dr. Rollefson: There are two ways of getting the N_2O molecule from N_2 and O. One is to activate the oxygen atom to the 1D state, the other is to have the system change electronic state as the oxygen atom approaches the N_2 molecule. The former would have the higher activation energy but the latter might have a less favorable probability factor.

Dr. Eyring: Both processes can no doubt be realized experimentally.

Dr. Rollefson: These calculations indicate whether or not a particular molecule can be formed although it may be destroyed rapidly by some other reaction. The triatomic halogens are unstable with respect to the reaction $2X_3 = 3X_2$, but that does not prevent having some X_3 molecules in a photostationary state.

Dr. Noyes: I have a rather naïve question. Mulliken and Hund, and others, have described electron configurations in terms of one electron

wave functions. Is one justified in giving any kind of minimum which predicts a stable molecule which violates the Pauli exclusion principle?

Dr. Eyring: Certainly not. One should never use eigenfunctions which are inconsistent with the Pauli principle. The methods of calculation which we have used in constructing potential surfaces are exactly as correct in this respect as are those of Hund and Mulliken and are the correct ones for the atoms far apart while their approximation is the correct one for the united atom. We are at present combining the two schemes. This in fact simply amounts to considering polar states in addition to the strictly homopolar ones we have employed in the past.

Dr. Noyes: Should stable H_3 violate that principle?

Dr. Eyring: With eigenfunctions entirely correct as regards the Pauli principle one can obtain a minimum corresponding to H_3 as, for example, is shown by the approximate calculations of Eyring and Polanyi. Whether this minimum is real or is introduced by the necessarily approximate calculations is not certain at the present time. The Pauli principle does not preclude the existence of such a minimum, however.

Dr. Rollefson: Most objections raised to this type of calculations are directed at the approximations involved in the numerical calculations rather than the nature of activation energies.

Dr. Eyring: At the present time Hirschfelder, Rosen and I are evaluating the triple exchange integrals for the linear H_3 molecule. These have previously been neglected. The calculation of exact surfaces is a very laborious procedure and for answering many important questions the easily constructed approximate surfaces serve almost as well.

Dr. Bates: As to the quenching of NO. You took only the case of the transition from 6^3P_1 to 6^3P_0 . If the transition is to 6^1S_0 , what do you obtain?

Dr. Eyring: I have not treated this transition explicitly. In principle it is essentially the same as that treated in the discussion of N_2O for which we have already given the transition probabilities. Thus a 1D O atom has a definite probability when colliding with a N_2 molecule of jumping to the 3P level and dissociating into a normal N_2 and a 3P atom. The corresponding potential curves for the Hg case could be drawn and the slope and interaction energy, calculated to obtain the probability of transition. Because the principle quantum number of the Hg changes M_{12} would now involve a potential due to the electrical as well as the magnetic dipoles. London has pointed out some of the inaccuracies in such a calculation. Greater accuracy is obtained

if we include both our electric and magnetic dipole interactions in our original Schrödinger equation. This then gives us non-crossing potential curves and we must now use as the perturbing potential responsible for transitions only a term depending on the slopes and curvatures of the surfaces in this region and on the relative collision velocity.

One other point could perhaps be made clearer. Consider the $6^3P_1 \rightarrow 6^3P_0$ transition of Hg during a collision in which the energy is taken up in the vibration of the colliding molecule. The magnetic vectors in the Hg interact with the magnetic moments associated with the electron spins of the bonding electrons. Now as the vibrational quantum number of the bond increases the magnetic field on the Hg increases because the valence electrons, being on the average further apart, compensate each other's spin magnetic moments less completely. Thus for large vibrational quantum numbers this magnetic field approaches the large value of the molecule dissociated into atoms. Thus we see how the quadrupole magnetic moment associated with a chemical bond can interact with the magnetic vectors in Hg. Where the bonding electrons have orbital moments this effect must also be considered.

Dr. Forbes: What is the limit of the availability of your method of determining reaction rates as the reactions considered become more and more complicated?

Dr. Eyring: Although very complicated molecules may enter into reactions and there may be a whole series of successive changes, the rate is determined, for many reactions at least, by a single slow process. Even in chain reactions there are in general only a few critical processes which are rate determining. In calculating a reaction rate we need to know only the properties of the original molecules and those of the critical complexes for the slow processes. Everything else is irrelevant. In seeking these slow processes we should use every possible chemical and physical consideration to eliminate unlikely mechanisms before resorting to the construction of potential surfaces for critical complexes. If all but a few of the possibilities can thus be eliminated the approximate surfaces we can now construct will often enable us to select between those remaining possibilities without excessive labor. However complicated the molecules, the fact that chemical forces fall off rapidly with distance insures that only a comparatively small number of atoms will participate in an important way in any particular slow process. The Boltzmann factor reduces to insignificance processes involving the breaking of more than a very few bonds simultaneously. Thus we probably will be able ulti-

mately to construct the important parts of the potential surfaces for even the more complicated reactions. I see no limitation in principle.

Dr. Forbes: Could one handle the quinine-chromic acid reaction, for instance?

Dr. Eyring: I think the general procedure of constructing the best possible potential surface for the probable slow processes together with calculations of the absolute rate for these processes is certain to shorten greatly the experimental journey toward a perfect understanding of such a reaction.

Dr. Rollefson: I would like to ask Dr. Eyring about one particular example. If you consider the reactions of atomic hydrogen with bromine and with hydrogen bromide, these calculations indicate a difference in the heats of activation of approximately eight large calories, yet experimentally there is no difference. To what do you attribute this discrepancy?

Dr. Eyring: This is a good illustration of the approximations of our present calculations. At the present time we cannot hope to calculate activation energies closer than to a few large calories, but even the accuracy available is sufficient to choose among several apparently possible mechanisms. Besides this, however, the fact that the force constants and the distances between atoms in the activated complex as given by these surfaces are nearly correct enables us to calculate the absolute rates (steric factors and collisions diameters) of reactions with an accuracy not suspected before the advent of such surfaces. The discrepancy pointed out by Rollefson is not unavoidable in principle, of course, since if one assumes a larger percentage of coulombic binding it disappears. However, it does indicate the need for continuing to improve our methods of calculating surfaces.

THE THEORY OF PHOTOACTIVATION AND THE PROPERTIES OF PHOTOACTIVATED MOLECULES

G. K. ROLLEFSON

In discussing the problem of photoactivation it is necessary to bear in mind the fact that the reactive state of a molecule produced by the action of light may be the same as that produced by thermal activation or it may be quite different. Thus the photochemical and thermal reactions between hydrogen and bromine to form hydrogen bromide apparently involve the same mechanism, whereas the corresponding reactions between hydrogen and chlorine proceed by different mechanisms. In general it may be said that photoactivation is less efficient than thermal activation in the sense that the amount of light energy absorbed in producing the reactive states is usually considerably larger than the heat of activation as determined from a study of the thermal reaction. Furthermore there are many reactions known in which activation energy in addition to that supplied by the light absorbed must be acquired. One of the most marked examples of this type is the reaction between hydrogen and bromine in which the bromine atoms formed by the photodissociation of bromine molecules require approximately 17000 calories per mol in order to react with the hydrogen.

Let us consider first the change produced in a molecule by the absorption of light and then proceed to a consideration of the follow processes. From the viewpoint of a physicist the absorption of light may change the rotational energy of the molecule, it may change the rotational and vibrational energies, or it may alter the electronic configuration of the system with simultaneous changes in rotational and vibrational energy. The last of these is the only one of any importance in photochemistry. For convenience in discussing such a change let us refer to Fig. 1 in which the potential energies of different electronic states of the molecule AB are represented as a function of the separation of A and B. In order to give a complete representation of the change of potential energy with r we should use a three dimensional diagram in which the system could be represented by a point moving on the surface. For diatomic molecules these surfaces may be formed by rotating a diagram such as that shown in Fig. 1 about the potential energy axis, but for more complex systems the slope of the surfaces at any point will depend not only on r but also on the orientation of A with respect to B. In our figure the molecule at ordinary temperatures may be considered as oscillating between the points x and y along the curve a .*

* In complex systems this oscillation will normally occur in such a manner that $d(PE)/dr$ is a minimum.

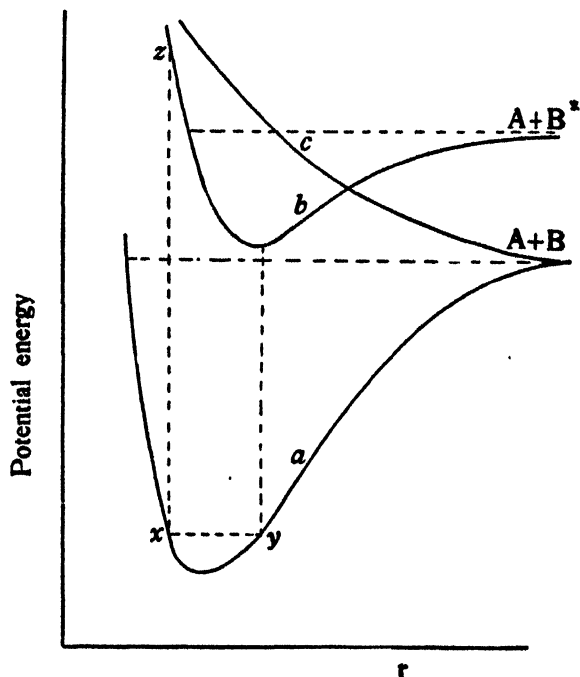


FIGURE 1

If the amplitude of vibration were gradually increased until dissociation occurs A and B would be formed; this is also true for curve c but for b the products are A and B^* where B^* means that B is in one of its higher electronic states. Now if the molecule absorbs light, according to the Franck-Condon principle it changes from curve a to b or c without any appreciable change in r . The frequency of the light required to produce such a change depends upon the separation of the curves representing the initial and final states, the quantitative relation being $h\nu = E_2 - E_1$. The light-energy which is absorbed by the molecule is therefore determined by the energy differences between the various electronic states and is in no way associated with the minimum energy which the molecule must acquire in order to take part in a reaction.

Once the molecule has been put into one of its higher electronic states by the absorption of light we have a competition between a number of reactions. One of these is the reverse of the activation process, i.e. fluorescence. Extensive study of fluorescence in gaseous systems has shown that the time that a molecule stays in the excited state before returning to a lower state with the emission of light varies considerably for different substances but is usually between 10^{-7} and 10^{-8} sec.

if fluorescence is observed. Some examples of longer life periods are 2.5×10^{-6} sec. reported by König and Ellett¹ for the 5^3P_1 state of cadmium and 1×10^{-5} sec. found by Heil² for the NO_2 molecule. That many other states of long life period with respect to fluorescence exist may be calculated from the transition probabilities as determined from absorption coefficient measurements, but in such cases other processes usually occur before the emission of light. In all such systems we must keep in mind that the time between collisions in gases at ordinary pressures is of the order of 10^{-9} sec. so fluorescence can be

the molecules are sufficiently complex so that we may assume that any active molecule produced by the action of light is deactivated before it has had many collisions with other molecules. The deactivation process need not be complete with one collision if there are any intermediate states available. Thus in the example we have cited, Hg in the 6^3P_1 state, many molecules first transfer the Hg to the 6^3P_0 state* and then after more collisions to the lowest state, 6^1S_0 .

In considering reactions involving activated molecules it must be remembered that the rate of reaction is proportional to the collision number,

TABLE I

Gas	H ₂	O ₂	CO	CO ₂	H ₂ O	N ₂	A	He
P $\frac{1}{2}$ mm.	0.2	0.35	0.4	2.0	4.0	30	240	760
Relative Efficiency	0.7	1.0	0.8	0.2	0.1	0.013	0.002	0.0003

observed only if the excited molecule is not affected by the number of collisions which occur in the life period. An estimate of the number of collisions which an excited molecule may suffer without undergoing a change is obtained from studies of the quenching of fluorescence. As a simple example let us consider the effect of various gases on the 6^3P_1 state of mercury. Table 1³ lists the pressures of the indicated gases at which the fluorescence is reduced to one-half its original intensity. At such pressures the probability of radiation occurring is equal to the probability of the deactivation process. The relative number of collisions under these conditions may be calculated by kinetic theory methods if we assume that the values for the molecular diameters based on other measurements may be used under these conditions. The third line of the table gives the relative efficiencies of the various molecules calculated in this manner. Thus if we assume that every collision with an oxygen molecule results in deactivation, one in five is effective with CO_2 , one in eighty with N_2 , and one in three thousand with He. We see, therefore, that the ability of an activated molecule to withstand collisions with other molecules depends to a very marked degree upon the nature of the colliding molecule. It is to be noted that the monatomic gases such as helium and argon which can remove energy from the photoactivated molecule only by acquiring kinetic energy have low efficiencies whereas the molecules which have the possibility of taking up energy as vibrational and rotational energy in addition to translational energy are much more efficient. In most reaction mixtures multiplied by the factor $e^{-Q/RT}$ where Q is what is called the heat of activation for the reaction. If the active molecules have a short life then Q

must be very small or else the molecule will return to its lowest state either by fluorescence or collision.

Up to this point we have been assuming that the molecule in the higher electronic state remains there (provided there are no disturbances due to collisions with other molecules) until it can return to the lowest state with the emission of light; this is frequently not the case. Returning to Figure 1 let us suppose that in absorbing light the molecule is transferred to the point z on the curve b ; then as r increases we find that the molecule separates into two parts with kinetic energy equal to the difference between the potential energy at the point z and the energy necessary for dissociation. A similar dissociation would occur if the molecule were transferred to any point on the curve c . The reality of such dissociations as a consequence of the action of light on molecules has been demonstrated by work on the alkali halides. Terenin⁴ showed that if sodium iodide, for example, were illuminated with light of such frequency that the energy of the quantum was sufficient to dissociate the molecule and excite the sodium atom to the 3^2P state the yellow doublet ($3^2S_{1/2} - 3^2P_{1/2}$, $3^2S_{1/2} - 3^2P_{3/2}$) appeared in fluorescence. Kondratjeff⁵ showed (using CsI) that this process occurred without the aid of collisions; and finally Hogness and Franck⁶ determined the Doppler width of the fluorescent spectrum lines and found that it corresponded to that calculated on the assumption that the excess of energy over that required to dissociate the molecule and excite the sodium

* 6^3P_0 is metastable and does not give rise to fluorescence.

atom appeared as kinetic energy of the separating parts. From the diagram in Fig. 1 it is evident that the time required for such a dissociation corresponds to one half of a vibration of the molecule. Various methods of estimating the time of a vibration agree on a figure of the order of magnitude of 10^{-13} sec. Under such circumstances there is no appreciable amount of fluorescence nor do the activated molecules take part in any collisions before they dissociate. The problem for the photochemist with such a system is to determine the nature of the free radicals formed and what happens to them in the follow reactions.

A third possibility for the behavior of the molecule after the absorption of light quantum which is intermediate to the two previously discussed may be illustrated by referring again to Figure 1. Consider that by absorbing light the molecule has been transferred to a state represented by a point on the curve *b* at which the total energy is less than that required for a dissociation into *A* and *B** yet equal to or greater than that corresponding to the intersection of curves *b* and *c*. In such a system there exists a finite probability (subject to certain selection rules) that a transition to the state represented by curve *c* will occur with resultant dissociation into *A* and *B*. The time elapsing before this transition occurs may be as short as the time of a vibration, 10^{-13} sec., or it may be so long that many of the molecules may return to a lower state with the emission of light before such a transfer has a chance to occur. If the dissociation occurs in 10^{-10} sec. or less the action of the light quantum may be represented by $AB + h\nu = A + B$, regardless of the mechanism involved. If a longer time elapses then it is necessary to consider the effect of collisions on the probability of passing from curve *b* to *c*. The best example we have of an effect of this type is the so-called "induced predissociation" in iodine vapor.

The absorption spectrum of iodine in the visible portion of the spectrum consists of a series of bands extending from approximately 6000 Å to a convergence limit at $\lambda = 4989.3$ Å and then a continuous absorption extending a little beyond 4500 Å. In terms of the potential energy diagram (Fig. 1) the bands correspond to transitions from *a* to points on *b* below the energy necessary for dissociation into *A* and *B** (in this case *I* and *I**) and the continuum corresponds to transitions to points above this dissociation limit. The position of *b* with respect to *a* for the iodine molecule is such that the photoactivated molecules formed by absorption of light in the band region have sufficient energy to dissociate into normal iodine atoms. A possible means for such a dissociation

to occur would be for the molecule to pass from the state represented by *b* to one of the type represented by *c*. The probability of such a transfer occurring in iodine must be relatively low as a fluorescence corresponding to a life period of 10^{-7} sec. for the active molecule is readily observed and furthermore the structure of the absorption spectrum is perfectly sharp.*

Such a probability may be altered by collisions or by a magnetic field. The fluorescence of iodine is quenched markedly by application of a magnetic field, a fact which may be explained by such a transfer. More conclusive evidence, however, has been obtained by Turner⁸, who showed that iodine atoms were formed when iodine vapor absorbed light in the band region and the number of atoms formed was increased by the addition of an inert gas. Similar conclusions were reached by Kondratjeff⁹ on the basis of a study of the effect of inert gases on the absorption coefficient of iodine.

If the light sensitive molecules are polyatomic the geometrical representation of the behavior of the molecules in terms of potential energy surfaces becomes difficult but the fundamental principles remain the same. It may be said in general that if a molecule has acquired energy sufficient to break some bond there exists a definite probability of that bond being broken. This probability may be so small that the dissociation process is of no importance or it may be so high that a molecule is dissociated for every light quantum absorbed. In most photochemical reactions the energy absorbed is more than enough to break a bond so it is not surprising to find that the results obtained are often best explained by assuming atoms or free radicals as resulting from the action of the light. A few examples of gaseous systems are known in which the photoactivated molecule is not dissociated but is believed to transfer its energy in a collision to some other molecule which dissociates. The most thoroughly studied example of this type is the dissociation of hydrogen molecules by mercury in the 6^3P_1 or 6^3P_0 states.¹⁰ Even here it is possible that the dissociation is brought about by the reaction $Hg^* + H_2 = HgH + H$ followed by the decomposition of *HgH*. However, Calvert¹¹ has

* Conclusions concerning the life of an excited state based on the sharpness of the structure in the absorption spectrum depend on an application of the Heisenberg uncertainty principle. This principle may be put in the form $\Delta E \times \tau \cong h$, or replacing ΔE by $\Delta h\nu$ and dividing by h , $\Delta\nu \times \tau \cong 1$. If we assume that a line at approximately 5000 Å 0.1 Å wide is classed as sharp, then the life of the excited state must be 10^{-10} sec. or greater.⁷

carried out similar experiments in which the mercury was replaced by xenon and found that hydrogen atoms were produced although intermediate compounds must be considered rather improbable.

The decision as to the nature of the active state produced by the light absorption is often very difficult to make. In order to illustrate the methods employed let us consider briefly (1) some photochemical reactions of bromine in which the problem is solely whether dissociation occurs or not; (2) the decomposition of aldehydes and ketones in which the products of the dissociation must be determined. The absorption spectrum of bromine is very similar to that of iodine, i.e. a series of bands converging to a limit at approximately $\lambda = 5000 \text{ \AA}$, and a continuous absorption extending to shorter wave lengths. From the spectrum alone we should conclude that in the continuum we have dissociation and in the banded region formation of excited molecules. Chemical evidence in support of the dissociation in the continuum is obtained from the fact that the thermal and photochemical reactions between hydrogen and bromine can be explained most simply by assuming atomic bromine as an active intermediate.¹² On the other hand Jost¹³ has found that this reaction proceeds just as readily with light absorbed in the bands as in the continuum which leads to the conclusion that also under these conditions bromine atoms are formed. Similar results have been obtained by Booher and Rollefson¹⁴ for the reaction between bromine and acetylene. Both of these reactions are too complicated to be suitable for some of the more simple tests concerning the primary step. For this purpose the reaction between platinum and bromine studied by Urmston and Badger¹⁵ is far more suitable. They found no difference in the reaction whether blue (continuous absorption) or yellow (band absorption) light was used. Furthermore they found that the rate of the yellow light reaction was independent of the distance of the illuminating beam from the platinum surface while the molecules in the state to which they are directly excited are confined to the region of illumination (as was shown by photographs of the bromine fluorescence at low pressures). Experiments were also performed at such low pressures that collisions could play no part in bringing about dissociation. The conclusion we reach is that in the region of discontinuous absorption we have a competition between fluorescence and dissociation with the latter predominating and only the atoms are involved in any follow reactions.

In dealing with the aldehydes and ketones we find that the absorption spectra for frequencies which are effective in producing decomposition show the diffuse structure characteristic of the

predissociation process. It is to be noted that although absence of diffuse structure cannot be taken as proof that there is no dissociation, the presence of diffuseness is definite evidence (at least for gaseous substances at moderate pressure) that a dissociation occurs within 10^{-10} sec. or less and therefore must be considered as the primary result of the action of light on the molecule. For the aldehydes and ketones the problem is to decide how the molecules decompose. At the present time we are faced with such discrepancies in the experimental data that it is not possible to claim a very definite proof for any particular mechanism. If we write the general formula for compounds of this type, R_1R_2CO where R_1 and R_2 are organic radicals in the ketones and R_2 is considered as H if we have aldehydes, it is generally agreed that the net result of the reaction is the formation of carbon monoxide and various hydrocarbons. There is considerable disagreement as to what hydrocarbons are formed and in what amounts. Kirkbride and Norrish¹⁶ state that the reactions proceed 90 percent according to the equation $R_1R_2CO + h\nu = R_1R_2 + CO$. Later work by Leighton and Blacet¹⁷ on the aldehydes indicates that the molecules R_1CHO decompose 80 percent or more to R_1H and CO and 20 percent or less to $\frac{1}{2}(R_1)_2$, $\frac{1}{2}H_2$, and CO. The decomposition of ketones has been studied by Norrish, Crane and Saltmarsh¹⁸, by Damon and Daniels¹⁹, and by Pearson²⁰. There is some disagreement as to analysis of the products but the results for unsymmetrical ketones as summarized by Pearson indicate the formation of carbon monoxide and approximately equivalent amounts of the possible hydrocarbons; thus methyl ethyl ketone gives carbon monoxide and equimolar amounts of ethane, propane and butane. In view of the differences in the experimental results it is not surprising that differences exist concerning the mechanism of the decomposition. One of the principal questions raised was whether free radicals are formed in the decomposition of the photoactivated molecule. The most direct attack on this problem was made by Pearson, who tested for the presence of free radicals with lead, antimony, and tellurium mirrors. He reported no free radicals in the decomposition of the aldehydes but obtained definite tests for such radicals in the decomposition of acetone, methyl ethyl ketone, and diethyl ketone. His experiments do not give any information concerning the amounts of free radical formed but merely that such substances were present in the reaction mixture. The failure to obtain any positive tests with the aldehydes is not conclusive as the experimental arrangement used was not especially suited to the detection of free radicals of short life. In the aldehydes we would be looking for

alkyl radicals and atomic hydrogen, but if the rate of reaction between these is fast compared to the rate of reaction of one alkyl radical with another then it is possible that in Pearson's experiments all the free radicals had been destroyed before they reached the metallic mirrors.

If we adopt one extreme view, namely, that free radicals are unimportant in the decomposition then we should expect R_1R_2 and CO to be the sole products. On the other hand if we consider the formation of free radicals to be essential then the products to be expected depend on the properties of the free radicals formed. Assuming that the ketone R_1R_2CO on photoactivation breaks up into R_1 , R_2 , and CO and also assuming that the specific reaction rates for all alkyl groups with similar or other groups are the same we should expect the resulting hydrocarbon mixture to be a composition corresponding to one mole of R_1R_1 , one of R_2R_2 , two of R_1R_2 . According to the latest experimental work the proportion of R_1R_2 is less than this so it becomes necessary to assume different reactivities. It is possible to account for the analyses obtained by Leighton and Blacet in this way for if we have a mixture of R_1 and H and the fastest reaction involving them is the formation of R_1H that will be the principal product with minor quantities of $(R_1)_2$ and H_2 . This is not necessarily the explanation for the predominance of R_1H in the products as it is possible that the decomposition may proceed in part by way of free radicals and in part by direct formation of R_1H and CO.*

A process of the latter type is most apt to occur with the aldehyde as the high mobility of the hydrogen atom could permit it to wander to join the alkyl group as that separates from the carboxyl group. On replacing the hydrogen atom with a less mobile alkyl group the probability of such a process occurring would diminish and it is quite likely that it may be neglected entirely with most of the ketones. When more quantitative data are available concerning the reactions of free radicals it will be possible to give a more exact interpretation of the behavior of the aldehydes and ketones. The preceding discussion has illustrated how an analysis of the final products of a reaction may be used to cast light on the nature of the products formed as a result of the "pre-dissociation" process.

In a large number of gaseous reactions the activating action of light is the production of atoms on free radicals. The behavior of such systems with regard to the rate law followed and the amount of reaction per quantum of light absorbed

is determined by the chemical properties of these radicals. If they are capable of reacting with other molecules with little or no energy of activation we may have long reaction chains set up and we would observe a high value for the quantum yield, and the temperature coefficient of the reaction will be small. On the other hand if a high energy of activation is necessary for reaction with other molecules the radicals may react readily with each other either in the gas phase or at the walls of the reaction vessel. In such a system the reaction chains will be short, the quantum yield small (even less than one frequently), and the temperature coefficient relatively high. A detailed discussion of the properties of these radicals and the chain processes involving them will be left for the other papers.

Let us turn to a consideration of some of the problems involved in the photochemistry of solutions. The principles are really the same as for gases but the application of these principles is much more complicated. In the first place the absorption spectra never show any rotational structure as the perturbations due to the surrounding molecules are great enough to completely blur any structure involving such small separations. It is, therefore, impossible to draw any conclusions concerning the type of active molecule produced on the basis of the presence or absence of such structure. A far greater difficulty is the uncertainty as to the nature of the absorbing molecule. If we are dealing with substances dissolved in very non-polar solvents such as carbon tetrachloride or the saturated hydrocarbons it is not unreasonable to assume (unless definite experimental evidence to the contrary exists) that the molecules are the same as exist in the gaseous state at the same concentration and that the action of light is the same as it would be in the presence of an inert gas instead of a solvent. Thus iodine is probably the molecule I_2 in such solvents and reacts to light essentially the same as in the gas phase as is shown by the similarity of the absorption curves. On the other hand, in polar solvents or in the presence of other solutes the situation is usually very much more complicated. The absorbing molecule is more or less solvated in such a solution; this effect may be such that we can write a definite formula for the solvate or it may be a clustering of an indefinite number of solvent molecules around the solute molecule (as in the hydration of ions in aqueous solution) or both effects may be involved. If other solutes are present we may have complexes formed which will act as the light absorbers. In either case it may be necessary to modify the picture of the action of light on the absorbing molecule from that which may be applied to the molecule in the gaseous state.

* If we accept Pearson's statement that there are no free radicals formed in the aldehyde decomposition, then the products should be only R_1H and CO.

Very few solutions show any fluorescence. Among the inorganic compounds it is found only with the uranyl salts and some of the rare earths; among organic compounds it is found only in those which contain certain "fluorophor" groups (benzene ring and pyron ring are examples). The life period of the excited state has been determined for a number of the organic dye-stuffs by Gaviola and found to be of the order of 5×10^{-9} sec. (On the other hand uranyl salt solutions show the relatively long life of 10^{-4} sec. In all of these the excitation is thought of as being located in such a part of the molecule that it is "protected" from action by the solvent. Studies on fluorescein solutions indicate that under the most favorable conditions there is a quantum of fluorescent light for almost every quantum which has been absorbed; in other words the solvent has practically no quenching action. If this picture is correct it necessarily follows that molecules in these states will be relatively insensitive to collisions with other solutes. The data available on the quenching action of various substances (especially ions in aqueous solutions) show that only one of a hundred or more collisions has any effect.

The chemical behavior of the molecule after the absorption of light involves the same possibilities in solution as in the gaseous state; the activated molecule may enter into a reaction with some other molecule or it may dissociate into radicals which will react with the other molecules present or with each other. In general it may be said that the same principles are to be used in analyzing the experimental results but the process is more difficult because of the complexity of the dissolved molecular species. As a particular example let us consider the photodecomposition of oxalic acid which is sensitized by uranyl salts. The nature of the light sensitive molecule in this reaction has been sadly neglected. Some people have assumed that only UO_2^{++} is involved, others that it is a complex involving UO_2^{++} and oxalate ion. Recent work at the University of California has shown that in solutions having a hydrogen ion concentration greater than 0.01 normal the results may be accounted for quantitatively by considering that the uranium is in the form of the ions UO_2^{++} , $\text{UO}_2(\text{OH})^+$, and $\text{UO}_2(\text{HC}_2\text{O}_4)^+$. All of these absorb light in the effective region of wave lengths but only that absorbed by the oxalate complex need be considered to account for the observed decomposition. As these solutions show fluorescence and the life period of the excited state is unusually long (10^{-4} sec.) the excited $\text{UO}_2\text{HC}_2\text{O}_4^+$ ions formed by the absorption of light must be considered as decomposing by a predissociation process. The products of this decomposition cannot

be specified on the basis of the data available; they may be carbon monoxide, carbon dioxide, and UO_2OH^+ (or UO_2^{++} and OH^-) or some intermediate stages may be involved.*

The data on other reactions involving uranyl compounds are too incomplete to determine the active molecule but it is quite probable that all of them involve complexes similar to those above. West, Muller and Jette²¹ and Pierce²² have attempted to show that collisions of the second kind between uranyl and oxalate ions cause the decomposition of the oxalate. There are a number of objections to using such a theory. In the first place the results of West, Muller, and Jette show that the quantum yield rises as the oxalic acid concentration is increased and reaches a limiting value at a fairly low concentration of the acid. If we consider the system as involving a competition between fluorescence and reaction then the variation of the quantum yield with the oxalate concentration is given by $\gamma = 1 / 1 + k/(\text{oxalate})$. The results referred to approach the limiting value too fast to fit this law. A second objection is that with a mechanism of this type we must either assume that all compounds of hexavalent uranium which may be present react the same or we should find more complex curves than have been obtained. If complex formation is denied then we are left without any means of accounting for the experimental fact that the absorption of light by solutions containing uranyl salts and oxalic acid is decidedly greater than that to be expected from the absorption shown by separate solutions of the uranyl salts and oxalic acid. Since the oxalate complex will account for all the observed facts satisfactorily without the assumption of any specific effect due to collisions of the second kind and its existence must be granted in order to account for some of the facts it is undesirable to bring in any additional assumptions such as the one discussed above unless it becomes absolutely necessary.

Biologists have been greatly interested in a large group of reactions which involve sensitization by certain dyestuffs, particularly those of the eosin family. In some of these the dyes bring about an oxidation process involving dissolved oxygen. Kautsky²³ has assumed that the excited dye molecule puts the oxygen molecule into a higher state (Bonhoeffer and Harteck²⁴ suggest a

* A possibility is that carbon dioxide is eliminated leaving $\text{UO}_2\text{HCO}_2^+$ which may decompose spontaneously or with the aid of another light quantum. If the latter is the actual fact then this mechanism would aid in accounting for the low quantum yield (approximately 0.60). A fact which may be considered as favoring this mechanism is that formic acid has been reported as one of the products in solutions in which the reaction has proceeded fairly well to completion.

¹/₂ state) which is more reactive than the normal state. Now there is not the slightest evidence that an oxygen molecule in any state which may be reached with aid of the energy available from the absorption of a quantum of visible light is any more reactive than the normal molecules, therefore it is well to investigate other more chemical mechanisms. Blum and Spealman²⁵ have presented some evidence for the formation of peroxides in irradiated solutions of eosin containing dissolved oxygen. The amount of peroxide found by them in some cases exceeded the amount of dye present so they concluded that hydrogen peroxide was the substance formed. It is not possible to draw any definite conclusions from their data concerning the mechanism of the formation of this peroxide but the fact that the concentration of the dye, determined spectrophotometrically, decreased as the peroxide content of the solution increased would indicate that a peroxy compound of eosin was an intermediate. In order to have the peroxide concentration become greater than the original concentration of the eosin, it would be necessary to postulate a formation of the eosin peroxide followed by a splitting out of hydrogen peroxide with regeneration of the eosin or formation of some derivative of eosin which could form a peroxide. It would be particularly interesting to determine whether any quantitative relationship exists between the amount of eosin in solution and the maximum amount of peroxide which can be formed. This evidence would offer the possibility that the activated eosin molecule reacts with oxygen to form a peroxide which then reacts with some other substance. Such a mechanism is consistent with the general observation that peroxides react more rapidly than dissolved oxygen.

In the discussion of the preceding paragraphs it is not intended to deny the possibility of collisions of the second kind in solution, but rather to point out that existing data certainly do not exclude other mechanisms. It is virtually impossible to obtain positive proof that a given step in a mechanism involves only a transfer of energy and not a chemical reaction in the ordinary sense. Such a conclusion must rest upon the careful exclusion of the other processes, a fact which many people are prone to neglect. Progress in the field of the photochemistry of solution depends to a very marked degree upon the determination of the chemical nature of the substances in solution. We are not justified in assuming that a solution contains simple molecules of the substances used in making up that solution. The action of light in producing activated molecules or free radicals in solution follows exactly the same principles as for gaseous systems but the problems which we may classify as the chemistry of the system are far

more complex in solutions than in gases and are those which must be solved in the future.

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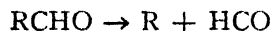
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DISCUSSION

Dr. Leighton: With respect to the mode of decomposition of aldehydes and ketones, the work of Leermakers¹ in demonstrating the existence of a chain photolysis in acetaldehyde at temperatures above 80° C., in which the rate is proportional to the square root of the absorbed light intensity, gives definite evidence that free radicals are produced in the primary process of the aldehyde decomposition. In this regard, we have some unpublished data on n-butyraldehyde which show a yield of hydrogen definitely increasing with decreasing wavelength, from about 1% H₂ (with

¹J. A. Leermakers, *J. Am. Chem. Soc.*, **56**, 1537 (1934).

respect to the amount of CO formed) at λ 3130 to over 30% H_2 at λ 2537. This would appear difficult to explain without some hypothesis involving the production of free radicals. The suggestion of Norrish², that the primary process in the aldehyde photolysis:



is followed rapidly by a spontaneous decomposition of the HCO radical into a hydrogen atom and a normal CO molecule, can be applied to explain the increase in hydrogen at shorter wavelengths if one assumes that as the wavelength is decreased a progressively larger number of the HCO groups become entirely separated from the alkyl radicals before they decompose into $H + CO$ or before a movement of the hydrogen from the carbonyl to the alkyl group can occur. This might be expected to follow from the greater kinetic energy which accompanies the dissociation at shorter wavelengths.

The complexity of the experimental results on the aldehyde and ketone photolyses would indicate a rather complex set of reactions, and the differences of opinion which Professor Rollefson mentioned are probably due in part to a tendency to explain these processes in terms of somewhat over-simplified mechanisms.

I should like to raise the question as to how far the principle, that purely continuous absorption necessarily produces dissociation, can be applied to complex molecules. Norrish, Crone, and Saltmarsh³ have explained the low quantum yield (0.4) in the continuous absorption region of acetone by the suggestion that absorption, even though in a purely continuous region, is followed in 60% of the cases by an internal rearrangement of the absorbed energy which prevents dissociation. Low quantum yields for the two butyraldehydes, the absorption bands of which show no perceptible structure and very little fluorescence, may be explained in the same way, but the lack of structure in absorption may be due in part at least to an overlapping of closely spaced bands.

A more striking case is furnished by crotonaldehyde, in which the characteristic carbonyl absorption band resembles that of the simpler aldehydes in showing distinct fine structure on the long wavelength side, merging into continuous absorption on the short wavelength side. Blacet and Roof have shown, however, that crotonaldehyde differs sharply from the simpler aldehydes in that it *cannot be perceptibly decomposed* by absorption anywhere in the band. To explain this total lack of decomposition, in spite of the

continuous absorption, it appears necessary to assume either a complete internal rearrangement with no perceptible dissociation, or else that a 100% efficient recombination process occurs.

Dr. Rollefson: With reference to the change in the relative amounts of hydrogen produced as the wavelength is changed, we can arrive at a possible explanation if we consider that in a diagram such as that in Figure 1 we have two or more curves of the type *c* crossing one of the type *b*. If one of these curves represents a state which dissociates into free radicals (which may yield hydrogen) and the other a state which passes adiabatically into the hydrocarbon and carbon monoxide, we will have a competition existing between three processes, fluorescence and the two decompositions. Now the intersections of the two *c* type curves with *b* will usually not coincide and therefore the relative probabilities of the transitions to what we may call c_1 and c_2 will depend on the point on the *b* curve which is reached by the excitation process. Therefore as the exciting wavelength is varied, the relative numbers of molecules transferring to the states represented by c_1 and c_2 will vary, resulting in large amounts of hydrogen being produced at some wavelengths and small amounts at others.

Dr. Kistiakowsky: I have some difficulty visualising how the mechanism suggested by Norrish for the deficient quantum yields can lead to diffuse spectra. After all, it does not matter what are the intermediate states. If the initial and the final states of the molecule are stable quantised levels, the absorption spectrum should be discrete. It may appear that one should expect a discrete spectrum from those molecules which do not decompose and diffuse spectrum from those which do. However, it seems to me, we are not justified in thus dividing the molecules. Rather, one should simply state that the probability of dissociation is, let us say for an imaginary case, equal to the probability of stabilisation. This will shorten the mean life by approximately a factor of two and thus should result in something like doubling the line width. So little broadening will ordinarily not be observable. To obtain sufficient broadening one would have to assume, just as in ordinary predissociation, that the probability of dissociation is much greater than the probability of stabilisation, but thus the entire advantage of this point of view would be lost.

Dr. Kassel: I agree with Kistiakowsky. If the internal rearrangement leads to a stable state, the energy is sharply defined and the absorption line will be sharp. If the states before and after rearrangement have different energies, the rearrangement is impossible. If the second state can dissociate, and thus has an unsharp

² R. G. W. Norrish, *Trans. Far. Soc.*, **30**, 108 (1934).

³ Norrish, Crone, and Saltmarsh, *J. Chem. Soc.*, **1934**, 1463.

energy overlapping the first state, rearrangement is again possible. I can not believe one gains anything by going from one sharply defined stable state through a continuum to another stable state. If the absorption process ends in a stable state of definite energy, the absorption line must be sharp. The uncertainty principle may obscure the conservation of energy, but it never violates it, as would be done if the transition from a state of energy E_1 to one of energy E_2 were produced by absorbing any frequency other than

$$\nu = (E_2 - E_1)/h.$$

Dr. Noyes: I have an attitude which is somewhat pictorial, but after all the transition from one electronic state of the molecule to another involves change of a point on a complex surface to a point on another complex surface with a series of ridges and valleys and if one applies the Herzberg and Teller selection rules, vibrations excited in the upper electronic state will be symmetrical vibrations, that is, there is only one which need be considered here, involving variation in the angle between the carbon-carbon bonds. There will also be symmetrical carbon-hydrogen vibrations. When such a transition occurs some unsymmetrical vibrations may be excited because the selection rules may not be rigorously obeyed, and there may be a shift of the motion from one type to another. In so doing the point may wander over a ridge onto a slope which results in dissociation. One may have at one time discrete absorption and at another time diffuse absorption. However, the probability of dissociation will not be unity and one may have a quantum yield less than one in the primary process.

Dr. Kistiakowsky: Concerning the presence of underlying continuum in absorption spectra of aldehydes and ketones, I cannot answer this question generally; but in the case of formaldehyde, the whole spectrum of which we have recently photographed under very high dispersion, there is undoubtedly no continuum. What appears as continuum, in photographs taken with moderate resolving power instruments, is in reality a number of very faint bands which have as much structure as the others. Incidentally, I may add, the spectrum of formaldehyde begins to become diffuse already above 3000 Å. although some evidence of rotational structure is observable down to 2700 Å.

Dr. Brackett: The continuum would decrease at a higher rate than the line spectrum. The ratio would be as the square with increasing resolution.

Dr. Noyes: I think the situation in acetone is complicated by the disagreements in experimental results. In regard to fluorescence, Norrish states that no fluorescence occurs below 3000 Å., and Daniels found fluorescence as far down as 2537 Å. Our observations, although without high precision (probably we had some stray radiation) showed fluorescence as far as 2537 Å.

Dr. Bonhoeffer: In connection with the question of photodecomposition of aldehydes I would like to direct the attention to experiments performed and communicated at the Bunsengesellschaft 1935 by Patat and Sachsse who investigated whether the photochemical decomposition of aldehydes causes a transformation of parahydrogen in ordinary hydrogen if it is admixed to the aldehyde. Since they found but a small transformation, they concluded that the stationary concentration of hydrogen atoms during the photochemical decomposition is low and that it is very improbable that decomposition takes place by way of H-atom formation.

With regard to the photo-oxidation of organic compounds I would like to ask Rollefson whether he knows of experiments which definitely show that oxygen in the $^1\Sigma$ state is not chemically activated. I think it would be good to make experiments just to settle that question. But even if an activity were found, there would still remain our ignorance about the metastability of these states in solutions. The identification of the photoactivated state of oxygen assumed by Kautsky with $^1\Sigma$ is not proposed by Harteck and me, but by Kautsky himself after a communication with Mecke.

Dr. Rollefson: The experiments of Patat and Sachsse show that the concentration of hydrogen atoms in the photochemical decomposition of the aldehydes is low but that does not necessarily mean that hydrogen atoms are not formed in considerable numbers. For example, the photosynthesis of hydrogen chloride certainly involves hydrogen atoms yet the concentration of these atoms under the conditions usually prevailing in the study of that reaction is too low to cause any detectable transformation of parahydrogen. The fact that Patat and Sachsse found some transformation could be taken as evidence that the amount of hydrogen found in these decompositions may be attributed to the combination of the hydrogen atoms present.

With regard to Bonhoeffer's second question, there is no evidence concerning the chemical activity of the $^1\Sigma$ state of oxygen. (See also the discussion following H. S. Taylor's paper.)

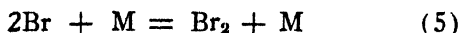
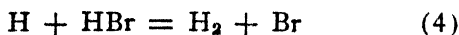
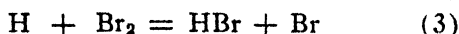
THE NATURE OF THE ACTIVATED STATE IN CHEMICAL KINETICS¹

LOUIS S. KASSEL²

It is now nearly fifty years since Arrhenius³ first wrote his famous reaction rate equation

$$k = Ae^{-E/RT} \quad (1)$$

and called E the energy of activation. For the greater part of that time physical chemists considered activated molecules to be sharply differentiated individuals—enol forms of ketones and aldehydes, alkylidene forms of olefins ($R_2C' - C'R_2$), addition complexes, dissociated radicals, atoms or ions, in short, distinct chemical species. The modern tendency in reaction kinetics, although it does not deny the intervention of such more reactive chemical species in many reactions, focusses attention also on the behavior of the Arrhenius-activated form. It is then usually found that the reaction rate of this form is again represented at least approximately by an equation of the type (1) with a value of E considerably greater than zero. As an example we may consider the reaction between hydrogen and bromine, which has been studied in great detail both thermally and photochemically by a number of workers⁴. The photochemical reaction may be assigned the following mechanism with complete confidence.



In the thermal reaction, (1), (5) and (6) are omitted and the concentration of bromine atoms is determined by the equilibrium constant

$$(Br)^2/(Br_2) = K$$

Except for this one change, the mechanism is the same as that for the photochemical reaction. In the Arrhenius sense, the process of activation is

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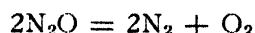
² Associate physical chemist, U. S. Bureau of Mines, Pittsburgh Experiment Station, Pittsburgh, Pa.

³ Arrhenius, *Z. phys. Chem.*, **4**, 226 (1889).

⁴ Bodenstein and Lind, *Z. physik. Chem.*, **57**, 168 (1906). Christiansen, *Kongel. Danske Videnskab. Selskab. Math.-fys. Medd.*, **1**, 14 (1919). Herzfeld, *Z. Elektrochem.*, **25**, 301 (1919). Polanyi, *ibid.*, **26**, 50 (1920). Bodenstein and Lutkemeyer, *Z. physik. Chem.*, **114**, 208, (1924). Jost and Jung, *ibid.*, **B3**, 83 (1929). Jung, *ibid.*, **B3**, 95 (1929). Ritchie, *Proc. Roy. Soc.*, **A146**, 828 (1934).

the dissociation of bromine molecules into atoms. It is found, however, that the rate constant for step (2) in this complex mechanism itself involves an activation energy of 17 kcal. At 250°C., where the rate of the thermal reaction is conveniently measurable, only one collision of Br and H₂ in 10⁸ results in reaction. The step (2) is known to be about 14 kcal. endothermic, but even when only collisions are considered with sufficient energy to make reaction thermochemically possible, it is seen that but one such collision in 50 is able to supply the additional activation energy needed. It is evidently unsatisfactory to identify the activated state with the bromine atom.

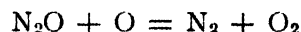
When the term activated molecule is no longer applied to reactive chemical species of the Arrhenius type, however, the question immediately arises to what it shall be applied. This problem can best be approached by considering the group of unimolecular reactions. A simple example, with a mechanism at least temporarily free from controversy, is the decomposition of nitrous oxide⁵ according to the chemical equation



At pressures of ten atmospheres or more and temperatures near 700°C. this is a reaction of the first order (that is, the rate is proportional to the pressure of nitrous oxide). It is generally accepted that the primary reaction is



followed by the rapid and for our present purposes less interesting step



From experimental results, the primary reaction has an energy of activation of 53 kcal. The accepted theoretical interpretation of the mechanism of this reaction is that molecules of nitrous oxide which have a total vibrational energy in their four degrees of freedom of at least E_0 (where E_0 is some characteristic quantity not greatly different from 53 kcal./mole) can react spontaneously in accordance with the equation



⁵ Hinshelwood and Burk, *Proc. Roy. Soc.*, **A106**, 284 (1924). Volmer and Kummerow, *Z. phys. Chem.*, **B9**, 141 (1930). Nagasako and Volmer, *ibid.*, **B10**, 414 (1930). Musgrave and Hinshelwood, *Proc. Roy. Soc.*, **A135**, 23 (1932). Volmer and Fröhlich, *Z. phys. Chem.*, **B19**, 85, 89 (1932). Volmer and Bogdan, *ibid.*, **B21**, 257 (1933). Volmer and Brücke, *ibid.*, **B25**, 81 (1934).

This spontaneous reaction is thought to occur when the more or less continuous redistribution of vibrational energy among the different degrees of freedom results in the accumulation of at least the critical energy E_0 in some one degree of freedom and the consequent rupture of the N-O bond. (It may be remarked in passing that the possibility of redistribution rests upon features of the internal potential energy function which make it impossible to assign with complete precision any distribution of energy to separate degrees of freedom. The picture of the reaction process given here must not be examined microscopically, but it is correct in all major features). The probability that this critical accumulation of energy will take place obviously increases rapidly as the total vibrational energy increases beyond E_0 . These higher energy molecules are thus rapidly removed by reaction. At the same time they are being both produced and destroyed by the redistribution of energy between two molecules at collision. These latter two processes if left to themselves would maintain an equilibrium concentration of the high energy molecules which can be calculated from the Maxwell-Boltzmann distribution law. Since the loss of high energy molecules by reaction constitutes an additional drain, the actual steady state concentration is less than the equilibrium concentration. This deficiency is greater the higher the energy of the molecules in question, due to the rapid increase in reaction rate with energy. It is also greater the lower the pressure, since the number of collisions a molecule makes, and therefore its chance of obtaining high energy, is proportional to the pressure. At high enough pressures the collision process is able to maintain essentially the equilibrium concentration of all molecules except those of extremely high energy, for which even the equilibrium concentration is very small. These very exceptional molecules make only a negligible contribution to the total reaction, and therefore at high enough pressures the reaction is first order. As the pressure is lowered, the steady state concentration of molecules in the important energy range falls at first gradually, then more and more rapidly, below the equilibrium concentration, and the rate of reaction falls more and more below that predicted by extrapolation of the high pressure first order rate. When low enough pressures are reached almost every molecule which succeeds in obtaining the energy E_0 is given time to decompose before making another collision at which the energy might be lost. The rate of the reaction will then be proportional to the number of collisions, and hence in this pressure range the reaction is second order. The experimental results, not only on nitrous oxide, but on a group of several dozen

decompositions, agree in all their most prominent features with a relatively simple mathematical theory based on this mechanism of collisional activation and spontaneous decomposition.⁶ In the theory an activated molecule is unambiguously one with vibrational energy in excess of E_0 . In reality, however, things are not quite this simple. Quantum mechanical calculations based on a model with only two degrees of freedom have shown that, although the condition that a molecule be capable of decomposition is merely that it shall have energy greater than E_0 , the specific rate of decomposition depends to a marked extent on the way the total energy is distributed between the two degrees of freedom.⁷... There is no doubt that this dependence would be even more important for a model with more degrees of freedom. There is also experimental evidence on this point. The pressure at which a unimolecular reaction departs appreciably from first order determines the number of degrees of freedom in the model, subject to some uncertainty connected with the frequencies of the various vibrations. In some reactions the number of degrees of freedom in the model is about the total number possessed by the actual molecule; in a number of other cases it seems to be very much less, but in no single case is the evidence perfectly satisfactory. It seems very probable, however, that some reactions actually are of this latter sort, and that the degrees of freedom of the molecules involved are divided into two or more groups which can exchange energy with each other only very slowly⁸. A molecule with total energy E_0 may always react if given sufficient time, but at pressures not too greatly different from that at which the rate begins to fall away from first order, nearly all the reacting molecules will satisfy the more drastic condition of energy at least \bar{E}_0 in some particular group of degrees of freedom. This condition provides a satisfactory definition for an activated molecule in unimolecular reactions.

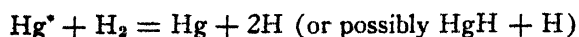
In the case of bimolecular reactions we encounter more difficulty. It is known that the possibility of reaction at collision depends greatly upon the orientation, and it is probable that relative kinetic energy is more important than either rotational or vibrational energy. There are certainly some bimolecular reactions which involve

⁶ O. K. Rice and Ramsperger, *J. Am. Chem. Soc.*, **49**, 1617 (1927); **50**, 617 (1928). O. K. Rice, *Proc. Nat. Acad. Science*, **14**, 114, 118 (1928). Kassel, *J. Phys. Chem.*, **32**, 225, 1065 (1928). For a general review, see Kassel, *Kinetics of Homogeneous Gas Reactions*, Chemical Catalog Company (New York) pp. 93-113.

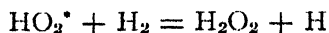
⁷ Rosen, *J. Chem. Phys.*, **1**, 319 (1933). O. K. Rice, *ibid.*, **1**, 625 (1933).

⁸ Cf. O. K. Rice, *Z. phys. Chem.*, **B7**, 226 (1930).

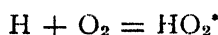
a definitely activated molecule. An indubitable example is



where Hg^* is produced by absorption of the mercury resonance line. More in the line of speculation is



where HO_2^* has been formed by



and thus possesses the heat of that reaction.⁹ These examples and others which might be given all have the characteristic that one partner in the reaction obtains high energy by a process which is not available to the other partner. In normal thermal reactions such a condition cannot be fulfilled and it can then be shown by simple calculations based on the Maxwell-Boltzmann distribution law that except in very special cases only a small contribution to the total reaction rate is made by collisions involving one molecule of such high energy that it could react with any other molecule of correct chemical species with which it might collide¹⁰. In general, reaction is conditioned by the energy of both molecules and the activated state must involve both.

It is possible in principle to define an activated pair of molecules in terms of their individual properties before the collision. Such a definition is not totally fruitless, but for our present purposes it proves of little value. It is more helpful here to give up the concept of activation as a condition with any duration in time. The situation is simplest for those reactions which involve no change of spin-multiplicity and which satisfy the other conditions of the Eyring-Polanyi theory¹¹. The quantitative validity of certain approximations customarily made in using this theory is debatable, but there is no reasonable doubt that the qualitative description of the reaction process is correct. All possible relevant states of both reactants and products may be represented by a generalized single-valued contour map in which total potential energy is

plotted against the coordinates. The state of the system is represented by a point on the map, and the process of reaction by the motion of this point over a pass or saddle-point. When the system is in the state represented by this saddle-point, the bonds which break in the reaction have been loosened, and the new bonds partially formed. The height of the pass is the critical energy which is necessary for reaction to occur, and is not greatly different from the statistically defined energy of activation. Molecular collisions which will lead to reaction are described by a variety of parameters, but from whatever point on the map they originate, they cross the pass not far from its lowest point. It is thus not unreasonable to consider the activated state to be merely the ridge perpendicular to the pass. This concept has some resemblance to the older, less precise idea of the activated complex which has played a prominent role in the theory of reactions in solution. It must be kept in mind, however, that this activated state is a configuration through which the reacting system passes, rather than a condition in which it exists. The failure to realize this distinction is a serious flaw in many attempted thermodynamic treatments of the activated complex.

When the spin-multiplicity changes during the reaction, conditions are more complicated. There will then be two superimposed potential energy surfaces crossing each other, one for each multiplicity. The system must make a transition from one to the other in order to react; this transition is made most easily along the intersection of the two surfaces. In some cases this intersection may play a role not too different from the ridge perpendicular to the pass in the preceding case, but in others there may also be a pass to be surmounted. Even in the most favorable cases there is a transition probability of the order of 10^{-3} for the change in multiplicity.

It is important to realize that we are permitted considerable freedom in defining the activated state. This freedom stands in sharp contrast to the situation regarding activation energies. There are just two energies which are of interest, one the minimum energy necessary for reaction, the other the excess of the average energy of the reacting molecules over the average energy of all molecules. There has been some confusion of nomenclature, but it seems desirable to call the former the critical energy, the latter the energy of activation, since it is the experimentally determined E in equation (1). We may, on the other hand, select any state of kinetic interest to call the activated state. At the present time this interest seems to the author to dictate the definitions that have been given here.

⁹ Kassel and Storch, *J. Am. Chem. Soc.*, **57**, 672 (1935).

¹⁰ R. H. Fowler, *Statistical Mechanics*, Cambridge University Press, p. 460. Kassel, *Phys. Rev.*, **35**, 261 (1930); *Kinetics of Homogeneous Gas Reactions*, pp. 62-68.

¹¹ Eyring and Polanyi, *Z. phys. Chem.*, **B12**, 279 (1931). Eyring, *J. Am. Chem. Soc.*, **53**, 2537 (1931). Pelzer and Wigner, *Z. phys. Chem.*, **B15**, 445 (1932). Wigner, *ibid.*, **B19**, 203 (1932). Ekstein and Polanyi, *ibid.*, **B15**, 334 (1932). Sherman and Sun, *J. Am. Chem. Soc.*, **56**, 1096 (1934). Eyring, *J. Chem. Phys.*, **3**, 107 (1935). Kassel, *ibid.*, July (1935).

DISCUSSION

Dr. Noyes: How much do you expect the energy of activation changes with the temperature?

Dr. Kassel: It is inconvenient to have it change very much with the temperature. Dr. O. K. Rice at one time had a theory according to which the energy of activation changed considerably, which he arrived at by combining classical and quantum theory elements in a way that he later abandoned for other reasons. A change of a few calories per degree—of the order of specific heats—is tolerable, but, at least in gas reactions, our present theories cannot account for more than that.

Dr. Rollefson: It seems to me that gas reactions have very little chance of having large variations in the heat of activation. Some idea of the effect may be obtained by considering a simple reversible reaction. The heat of the reaction is equal to the difference between the heats of activation, and we know that the change in the heat of reaction with temperature depends on the difference between the specific heats of the products and reactants, which is a very small change for gases. If the change in the heat of reaction is small, it is probable that the changes in the heats of activation will be small. In solutions the change of the heat of reaction with temperature involves partial molal heat capacities which often vary in such a way as to introduce large differences between the heat capacities of the reactants and products. Under such circumstances it would not be surprising to find fairly large changes in the heats of activation for the reactions involved.

Dr. Mestre: How far can the concept of the distribution of vibrational energy through the practically unlimited number of degrees of freedom of such very large molecules as proteins be carried?

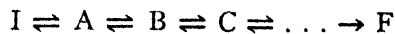
Dr. Kassel: I think you can carry it to molecules as large as you please. The thing one wants to remember, though, is that when you get very big molecules, the possession of a large energy in them does not necessarily produce any immediate result. Any large molecule containing several hundred atoms is, even at room temperature, frequently going to possess at least the energy of activation, and thus be capable of reaction. Unless the large molecule, however, has considerable energy in excess of this minimum requirement the reaction will be quite slow because when there are many degrees of freedom it is very unlikely that all the energy will accumulate in some single one.

Dr. Kistiakowsky: Is not this partly responsible for the relatively great instability of, let us say, protein molecules which contain essentially the same bonds as are present in small molecules but which decompose on heating to 50° or 80° C.? With small molecules heating to 200° to 300° leaves them unchanged.

Dr. Kassel: On the basis of the theories I have discussed it is quite possible that the larger number of degrees of freedom of complex molecules is partially responsible for their instability. The magnitude of the effect, however, is greater than can be accounted for on this basis alone.

Dr. Mestre: In the thermal denaturation of proteins values of E of the order of magnitude of 85 k. cal. are encountered, coupled with reaction rates which may be extremely high at temperatures as low as 65° C. A similar condition exists in the case of the swelling of complex carbohydrates, such as starch, in the presence of hot water. On the basis of Dr. Kassel's remarks above, some further explanation would seem necessary to account for these high reaction rates in large molecules at relatively low temperatures.

Dr. Kassel: I think that if any reaction, for example the denaturation of proteins, has an activation energy of 85 k. cal. and yet is able to take place rapidly at 60°-70° C., it cannot very well be a simple reaction. The rate of a simple unimolecular reaction cannot be much greater than $10^{16} e^{-E/RT} \text{ sec}^{-1}$, and this will not permit a rapid reaction at 60° C. if E is much greater than 30 k. cal. For bimolecular reactions the limit is nearly the same, being possibly a little lower. Some years ago I proposed a rather general complex mechanism to account for a similar difficulty in the dehydration of $\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$ (J. Am. Chem. Soc., 51, 1136, 1929). The essential feature of this mechanism is a series of intermediates between the initial and final states:



If all the intermediate reactions are reversible, and if the temperature coefficients of the forward steps are considerably larger than those of the reverse steps, the over-all temperature coefficient may be very much larger than that of any single step. I would be inclined to suggest a complex mechanism for any reaction, whether or not it involves biological materials, which has a high activation energy and a high rate at moderate temperatures. I would regard the type of complexity given above as an example to demonstrate that such a mechanism is possible, but I would not assume without other evidence that the actual complex mechanism was necessarily of that sort.

THE KINETICS OF PHOTOCHEMICAL REACTIONS FROM THE STANDPOINT OF CHAINS.

W. ALBERT NOYES, JR.

The concept of reaction chains seems to have been introduced into chemical kinetics by Bodenstein¹ who used it to explain the photochemical hydrogen-chlorine reaction. The entire subject has recently been reviewed exhaustively by Semenov².

For the purposes of this paper we will define a photochemical chain reaction as *one in which the final product is not formed in the primary step*. This definition may be considered by many workers as too broad, for nearly all (if not all) photochemical reactions would be included within its scope. It may be defended, however, on the ground of expediency for any definition based on quantum yield would necessarily exclude from consideration many reactions of the chain type for some step of which the constant for the reverse reaction happens to be greater than that for the forward reaction.

Thus we include as chain reactions such a reaction as that of ozone formation in which the primary process probably is the dissociation of molecular oxygen (O_2) followed by the combination of each oxygen atom with an oxygen molecule³, as well as the hydrogen-chlorine reaction. The former reaction in which two molecules of ozone are formed per absorbed quantum is generally considered to be one which obeys the Einstein Law of Photochemical Equivalence while the latter in which as many as 10^4 molecules of hydrogen chloride may be produced per absorbed quantum, is not. In neither case, however, is the final product of the reaction formed directly upon absorption of a quantum.

The primary process is to be discussed elsewhere in this symposium. To summarize briefly, primary processes may be divided into two categories: 1) *The production of activated molecules*. The fates of these activated molecules may, in turn, be of several types: a) they may lose their energy as radiation, resulting in fluorescence; b) they may dissociate into atoms or free radicals upon collision with other molecules; c) they may lose their energy (be deactivated) by collision without either dissociating or producing a new type of molecule; d) they may rearrange either through the agency of collisions or spontaneously,

thereby producing the final product. Our definition of a chain reaction would exclude from consideration anything which would result from c) and in general also from a) and d). 2) *Dissociation into atoms or free radicals*. Dissociation results either from absorption in a continuum or by the process of predissociation⁴. When such atoms or free radicals are produced subsequent reactions occur resulting, by definition, in a chain.

The above definition of chain reactions would include automatically all sensitized reactions in which one molecule absorbs and transfers energy by collision of the second kind to another molecule resulting in its activation. We shall limit the field, however, to those reactions involving chains following this secondary activation.

The chain reaction is necessarily governed by the laws of kinetics and, of course, of thermodynamics. Thus a long chain (one in which many molecules react per quantum absorbed) can only take place if the overall reaction involves a decrease in free energy. The converse of this statement is by no means true for all photochemical reactions taking place with a large decrease in free energy do not involve long chains.

The decision as to whether a given reaction is of the chain type is frequently difficult. By the definition given above if more than one molecule (either of the light absorbing substance or of more than one substance entering into the reaction) disappears per quantum absorbed we are confronted with a chain. However if the quantum yield is unity or less the reaction may still be of the chain type. There are several tests which one may apply in this case.

1) If the quantum yield varies with light intensity, while the concentrations of the reactants are maintained constant, a chain is necessarily involved. Such a variation is usually explained by the recombination of free radicals or atoms and the yield usually (probably always) decreases as the intensity increases. Lack of variation of yield with intensity is not, however, a guarantee of the absence of chains.

2) If the quantum yield varies markedly with pressure or concentration the reaction is usually of the chain type. Here again one must take account of the effect of concentration on the optical absorption, since pressure effects may lead to a broadening of the absorption lines. It is possible also that at high pressures where deactiva-

¹ Bodenstein, M., Z. phys. Chem., 85, 329 (1913).

² Chemical Kinetics and Chain Reactions by N. Semenov, The Oxford University Press, New York, 1935. Reviewed by G. B. Kistiakowsky in J. Am. Chem. Soc., 57, 963 (1935).

³ Warburg, O., Sitzb. Akad. Wiss. Wien, 1912, 216; 1914, 872; Vaughan, W. E. and Noyes, W. A., Jr., J. Am. Chem. Soc., 52, 559 (1930).

⁴ Henri, V. and Teves, M. C., Nature, 114, 894 (1924); Comptes rendus, 179, 1156 (1924).

tion may follow activation very quickly, the type of reaction may be different from that at low pressures.

3) A reaction whose quantum yield varies little or not at all with temperature *may* not be of the chain type. However such a variation with temperature is not proof of the existence of chains since absorption may be occurring from higher vibration levels of the ground state with a resulting higher probability of reaction.

4) For many chain reactions the walls and various impurities determine the length and nature of the chains. If variations due to these causes are found, little doubt can exist as to the chain character of the reaction.

While none of the foregoing tests of the existence of a chain mechanism with the possible exception of the fourth are conclusive, frequently one may arrive at a fairly definite conclusion by a combination of methods. There are several types of experiment which indicate definitely that chains exist if the results are positive, but here again lack of a positive result may not prove the absence of chains. We may list a few of the methods, all of which have as their object the proof that free atoms or free radicals exist in the reacting mixture.

1) Attempts have been made to prove the existence of free atoms or radicals by obtaining their characteristic absorption spectra. So far as we are aware no definitely positive result has ever been obtained by this method since during photochemical reaction the concentrations of such intermediates must be exceedingly low.

2) In certain types of photochemical reaction fluorescence due to intermediate groups is sometimes observed. In the photochemical dissociation of salt vapors, for example, the characteristic spectrum of the positive constituent is frequently observed.

3) Most free radicals and many free atoms possess net electron spins which make them paramagnetic. This type of compound has the property of causing the ortho-para hydrogen conversion to take place. The presence of free radicals in several photochemical reactions has been indicated by this method⁵.

4) Many organic free radicals have the property of removing lead or other metallic mirrors with the production of metallo-alkyl compounds. This method has been successfully applied to several photochemical reactions⁶.

⁵ See the abstract of a paper presented by W. West before the Division of Physical and Inorganic Chemistry at the New York meeting of the American Chemical Society on Tuesday, April 23, 1935.

⁶ See for example T. G. Pearson, J. Chem. Soc., 1934, 1718.

5) Since free atoms and radicals are lighter than the parent molecules from which they came, their presence may sometimes be indicated by an increased thermal conductivity of the gas mixture in which they are formed. This method has been used successfully to show the presence of hydrogen atoms in a mixture of hydrogen and mercury vapor illuminated by the resonance line of mercury⁷.

6) Occasionally the presence of free groups may be made evident by purely chemical means. Thus molybdenum trioxide is reduced to a blue oxide either by hydrogen or oxygen atoms⁸.

Once the decision has been made as to whether a given reaction is of the chain type, the postulation of a mechanism and the derivation of a rate equation, together with the testing of this equation constitute the next important steps in the understanding of a photochemical reaction. We will consider first reactions at constant temperature, leaving for a later section the effect of this variable.

Several different methods of propagating photochemical reaction chains have been suggested or occur to mind.

1) The original suggestion made by Bodenstein¹ was to the effect that the chain carriers in the hydrogen-chlorine reaction were electrons. Subsequent work by Bodenstein⁹ and others indicated that the chains must be propagated by other means. There seems to be no clear-cut case on record in which chains are continued by charged particles, although they may be initiated in this way¹⁰.

2) At various times the suggestion has been made that chains are propagated by activated molecules⁹, so-called energy chains. Frequently mechanisms of this type lead to rate equations in satisfactory agreement with experiment, but in most, if not all, cases other explanations are more plausible. There is nothing inherently absurd in the idea of energy chains, although activated molecules have relatively short lives and in addition would not be expected to survive many collisions. There seem to be few if any cases on record in which energy chains provide a really sat-

⁷ Senftleben, Z. Phys., 32, 922 (1925).

⁸ Phipps, T. E. and Taylor, J. B., Phys. Rev., 29, 309 (1927); for an attempted application to photochemistry see Mahncke, H. E. and Noyes, W. A., Jr., J. Am. Chem. Soc., 57, 456 (1935).

⁹ Bodenstein, M., Z. Elektrochem., 22, 53 (1916).

¹⁰ See Moore, H. R. and Noyes, W. A., Jr., J. Am. Chem. Soc., 46, 1367 (1924); Noyes, W. A., Jr., Trans. Faraday Soc., 21, 569 (1926); Pierce, W. C. and Noyes, W. A., Jr., J. Am. Chem. Soc., 50, 2179 (1928).

isfactory explanation of the kinetics of a photochemical reaction^{10a}.

3) There is a possibility that fluorescence or chemiluminescence would propagate a chain, providing the fluorescent radiation is absorbed by the reacting molecules. The phenomenon of "imprisonment" of radiation is important in experiments involving excited mercury¹¹, but does not play an important role in other photochemical reactions.

4) Practically all chain reactions are propagated by free atoms or radicals. These free groups react with normal atoms or molecules producing in turn other free groups and so on until a free group is removed by some process.

Since the only method of propagating chains which we need consider seriously is that given under 4) above, we must next pay some attention to the methods by which the chains may be stopped. As already stated unless each step in the chain has an appreciable probability of taking place (in general this means being exothermic as well as having a low energy of activation) the average chain length will not be long. The chains may be stopped by several different methods.

1) Homogeneous recombination of free radicals. In the gas phase, for obvious reasons, such a recombination can usually take place only in the presence of a third body which permits the energy of combination to be dissipated. In a few cases this energy may be lost by radiation, but no clear-cut cases of this seem to be on record. In this chain stopping step the rate will be proportional to the product of the concentrations of the two bodies taking part as well as to the concentration of molecules which may play the role of third bodies. The latter concentration may *usually* be considered as constant.

In solution the solvent molecules are so numerous that no difficulty is experienced in providing enough three body collisions. However, relatively little is understood of the mechanisms of photochemical reactions in solution and the application of kinetic theory to such reactions is in a rudimentary stage.

2) Removal of atoms or free radicals by the walls. This process is undoubtedly important in gas reactions at low pressures, but is less apt to be so at moderate or high pressures unless the

chains are long. The first step is presumably the adsorption of the free group on the wall followed by its combination on the wall with another free group. In the derivation of rate equations it is generally assumed that the first step is slow compared to the second and that the rate of removal is proportional merely to the first power of the concentration of the free group involved. It may be pointed out, however, that this is not necessarily true and that erroneous conclusions as regards mechanism may be arrived at by making this assumption. The nature of the wall surface is of importance in thermal chain reactions¹² and would doubtless be important also in photochemical chain reactions.

3) Removal of atoms or free radicals by reaction with some added substance or with some impurity. If the concentration of the foreign substance remains approximately constant during the reaction, the rate of removal of the free group may be taken as proportional to its concentration. Otherwise an apparently complex reaction mechanism may result. The importance of oxygen as an inhibitor in the hydrogen-chlorine reaction has been known since very early studies on this system. Presumably its main effect is in removing hydrogen atoms so that the rate of the chain stopping step would be proportional to the first power of the concentration of hydrogen atoms and to the pressure of the oxygen. However, other factors enter the picture in this reaction and probably silicon compounds may also be effective in stopping the chains¹³. In long chain reactions it is frequently a matter of some difficulty to ascertain just what the chain breaking mechanism really is.

The decision as to which mechanisms are responsible for propagating and for breaking chains must be based on several different factors, but a study of the kinetics of the reaction alone rarely suffices to give an unambiguous answer to these questions. A knowledge of the behavior of the various intermediate free atoms and radicals would be of immense help in determining an over-all mechanism. Thus it is known that either hydrogen atoms¹⁴ or chlorine atoms¹⁵ will initiate chains in the hydrogen-chlorine reaction. While this by no means proves the correctness of the Nernst chain mechanism, it does make two of the steps in that mechanism plausible.

^{10a} See Heldt, L. J. and Forbes, G. S., *J. Am. Chem. Soc.*, **56**, 2363 (1934) who state that energy chains must be important in photochemical ozone decomposition.

¹¹ The theory has been discussed by Milne, E. A., *J. London Math. Soc.*, **1**, 1 (1926). See A. C. G. Mitchell and M. W. Zemansky, *Resonance Radiation and Excited Atoms*, The Cambridge University Press, Chapter IV, 1934 for a complete discussion.

¹² Cf. Pease, R. N., *J. Am. Chem. Soc.*, **52**, 5106 (1930).

¹³ Bodenstein, M. and Unger W., *Z. phys. Chem.*, **11B**, 253 (1931).

¹⁴ Taylor, H. S. and Marshall, A. L., *Nature*, **112**, 937 (1923); Marshall, A. L., *J. Phys. Chem.*, **29**, 842 (1925).

¹⁵ Rodebush, W. H. and Klingelhoefer, W. C., Jr., *J. Am. Chem. Soc.*, **55**, 130 (1933).

In some few cases the heats of reaction of free groups with various molecules may be estimated. For such simple steps as those in the Nernst mechanism for the hydrogen-chlorine reaction the data are sufficiently accurate and unambiguous to permit the calculation to be made with high precision for each step. Reactions which are decidedly endothermic would take place to a very small extent, but steps which are slightly endothermic or exothermic need not take place rapidly even if the heat evolved is quite large. The really important quantity in this connection is not the heat of reaction but the energy of activation. Another factor, sometimes called the "steric" factor is probably also of importance, although the order of magnitude of this factor does not seem to change much from one reaction to another in simple reactions. Some progress has been made in recent years in estimating the energies of activation for individual steps, but in most cases the results are little better than guesses. It is possible, however, to estimate the relative magnitudes of energies of activation for certain simple reactions¹⁶.

In the absence of specific information concerning each step one must rely on common sense to derive rate expressions. It must always be kept in mind, however, that agreement of a given rate expression with the data by no means proves that the mechanism upon which the equation is based is correct.

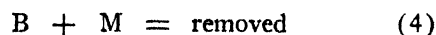
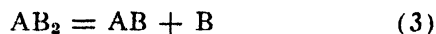
We will next examine the methods of deriving rate expressions and point out some of the difficulties in applying them rigorously to chain reactions.

It is nearly always assumed in deriving rate expressions that one is dealing with the steady state. That is the rate of formation of each intermediate free radical or atom is set equal to its rate of disappearance. Only with this assumption is it possible without a great deal of labor to eliminate the concentrations of these intermediates from the final rate expression.

As regards free atoms and free radicals the assumption of the steady state would seem to be, in general, justified. The life times of such groups do not exceed 10^{-2} or 10^{-3} second under ordinary conditions and frequently seem to be much less. Unless the rate of the over-all reaction is very high, due either to high light intensity or to very long chains, the rates of disappearance of these intermediates must rapidly become equal to their rates of formation. In some cases it is wise to examine the question more closely, how-

ever. If the rates of individual steps are known, one can form an estimate of the time necessary to arrive at a steady state. Otherwise one can sometimes ascertain from the shape of the various concentration-time curves whether the assumption of a steady state is justified. Induction periods are sometimes encountered in photochemical reactions. In general these may be ascribed to two causes: 1) The building up at the beginning of the reaction of some intermediate compound whose rate of disappearance is relatively slow. We will return to this point later, but we may mention in passing that this case is probably not unusual in complex reactions and it is often difficult to arrive at any theoretical treatment in such cases. 2) The presence of impurities. Sometimes these impurities are removed by reaction during the early stages, the full rate not being attained until after such removal is complete. Thus oxygen strongly inhibits the addition of chlorine to tetrachloroethylene¹⁷, a sensitized oxidation taking place until the oxygen has disappeared, following which the normal reaction occurs. In this case the oxidation is proportional to the light intensity, whereas the addition reaction has a rate proportional to the square root of this quantity. It seems probable that nearly all cases of true induction periods may be explained in this way. If the rate of a photochemical reaction starts out from zero time and follows a smooth curve without inflection points or irregularities one is reasonably safe in assuming a steady state as regards intermediate free radicals and atoms.

Let us consider a hypothetical reaction and determine the difference in behavior to be expected on the one hand if a steady state is rapidly reached, and on the other hand if it is not. In what follows we will use a somewhat unusual, but convenient, method of expressing the intensity of the radiation absorbed. By I_a we will denote the number of quanta absorbed per cubic centimeter per second. This quantity will be proportional to the intensity incident upon the vessel only if the concentration (and temperature) of the absorbing material remains constant, and it will vary from point to point in the light path even if the radiation is strictly parallel. Assume the following series of reactions



¹⁶ Eyring, H., *Naturwissenschaften*, **18**, 914 (1930); Eyring, H. and Polanyi, M., *Z. phys. Chem.*, **12B**, 279 (1931). Also a series of papers during the past three years by Eyring and his coworkers in *J. Am. Chem. Soc.* and *J. Chem. Phys.*

¹⁷ Leermakers, J. A. and Dickinson, R. G., *J. Am. Chem. Soc.*, **54**, 4648 (1932); Dickinson, R. G. and Leermakers, J. A., *ibid.*, **54**, 3852 (1932); Dickinson, R. G. and Carrico, J. L., *ibid.*, **56**, 1473 (1934).

The fourth step is introduced in this way to avoid a square root of the light intensity. M may represent the walls or a foreign molecule whose concentration is supposed to remain constant during the course of the reaction. Let the volume of the reaction vessel be V , the area of the light beam be A and the length of the absorbing column l . Now for the sake of simplicity we will assume a low absorption coefficient for A_2 , so that in the path of the light beam the number of quanta absorbed per cubic centimeter is constant. If the photochemical reaction is slow compared to diffusion processes, so that the concentrations of all molecules except the intermediates may be considered to be uniform, we may set up the following equations:

Twice the number of molecules of A_2 dissociating per second = the rate of production of A atoms = $2 I_a A l$ (5)

The number of molecules of AB_2 formed per second (assumed to be in the light path only) = $k_2 A l C_A C_{B_2}$ (6)

where C_A and C_{B_2} are the numbers of molecules of A and B_2 respectively per cubic centimeter in the light path.

The number of molecules of AB formed per second = $k_3 A l C_{AB_2}$ (7)

assuming that the intermediate AB_2 does not have time to diffuse out of the light path.

The rate of removal of B atoms will be (making the same assumption) = $k_4 A l C_B C_M$ (8)

If the steady state is now assumed for all intermediates, we obtain the following equations:

$$\begin{aligned} 2 I_a A l &= k_2 A l C_A C_{B_2} ; \\ C_A &= 2 I_a / k_2 C_{B_2} \end{aligned} \quad (9)$$

$$\begin{aligned} k_2 A l C_A C_{B_2} &= k_3 A l C_{AB_2} ; \\ C_{AB_2} &= k_2 C_A C_{B_2} / k_3 = 2 I_a / k_3 \end{aligned} \quad (10)$$

$$\begin{aligned} k_3 A l C_{AB_2} &= k_4 A l C_B C_M ; \\ C_B &= k_3 C_{AB_2} / k_4 C_M = 2 I_a / k_4 C_M \end{aligned} \quad (11)$$

If the experimental method used for determining the rate detects the quantity of AB formed, the rate will be

Number of molecules of AB formed per second = $2 A l I_a$ (12)

Since the volume of the vessel is V , we may write

$$+ d C_{AB} / dt = 2 A l I_a / V \quad (13)$$

The type of chain referred to in equations 1 - 4 is, of course not self-propagating and the rate is independent of the concentration of B_2 and de-

pends only on the concentration of A_2 in so far as the light absorbed depends on this quantity.

If the experimental method for determining the rate is based on the quantity of A_2 disappearing (as for example by measuring its concentration through optical absorption) we find

$$- d C_{A_2} / dt = I_a A l / V \quad (14)$$

The rate of disappearance of B_2 is

$$- d C_{B_2} / dt = 2 I_a A l / V \quad (15)$$

If, now, we are permitted to assume the steady state only as regards the atoms A and B , but not for the intermediate AB_2 , the rate of formation of AB becomes

$$+ d C_{AB} / dt = k_3 C_{AB_2} \quad (16)$$

However C_{AB_2} must be calculated from the differential equation

$$+ d C_{AB_2} / dt = 2 I_a A l / V - k_3 C_{AB_2} \quad (17)$$

If the light absorbed is proportional to the concentration of A_2 we may write $I_a = K C_{A_2}$ and

$$- d C_{A_2} / dt = K C_{A_2} A l / V. \quad \text{Hence}$$

$$C_{A_2} = C_{A_2}^0 \exp (- K A l t / V) \quad (18)$$

where $C_{A_2}^0$ is the concentration of A_2 at the beginning of the reaction. Substituting in equation (17) we obtain

$$\begin{aligned} + d C_{AB_2} / dt &= 2 K A l C_{A_2}^0 \exp \\ &(- K A l t / V) / V - k_3 C_{AB_2} \end{aligned} \quad (19)$$

Integration gives

$$\begin{aligned} C_{AB_2} &= (2 K A l C_{A_2}^0 [\exp (- k_3 t) - \\ &\exp (- K A l t / V)] / (K A l / V - k_3) \end{aligned} \quad (19a)$$

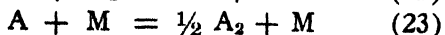
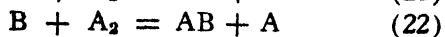
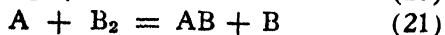
The rate of formation of AB will be $k_3 C_{AB_2}$. The rate of formation of AB will start at zero at the beginning of an experiment and will increase for a length of time dependent upon the magnitudes of the various constants until the rate of formation of AB_2 is equal to its rate of disappearance. In case its rate of disappearance at unit concentration is small compared to its rate of formation when A_2 and B_2 are at unit concentration, the rate may continue to increase until A_2 and B_2 have nearly disappeared.

In the case just cited the distinction between the case in which the steady state may be assumed and the one in which such an assumption is not justified is not clear-cut, but will depend entirely on the relative values of the reaction constants. The second method of solution resulting in equation (19a) is, of course, rigorous and will give the same answer as the assumption of the steady state when that assumption is justified.

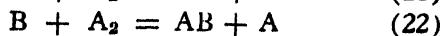
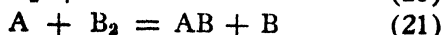
One further point may be emphasized. The quantum yield may be defined either in terms of the number of molecules formed or in terms of the number of molecules disappearing per quantum absorbed, and hence may be obtained by dividing any one of equations 13, 14 or 15 by $I_a A l$, the number of quanta absorbed per second, and multiplying by the volume. The quantum yields are all, therefore, independent of the light intensity.

Now let us consider two examples of continuing chains.

Mechanism I



Mechanism II



By making the same postulates as before one finds

$$+ d C_{AB}/dt = (I A/V) \cdot 4 I_a k_2 C_{B_2}/k_4 C_M = 2 d C_{A_2}/dt \quad (25)$$

for Mechanism I. Since the number of quanta absorbed per second is $I_a A l$, the quantum yield is

$$(V/I_a A l) d C_{AB}/dt = \frac{4 k_2 C_{B_2}}{k_4 C_M} \quad (26)$$

The quantity $k_4 C_M$ determines the rate of removal of atoms of A and may be either a wall reaction or a homogeneous reaction without changing the general form of the equation. If the light absorbed is $I_a l = I_0 [1 - \exp(-a C_{A_2} l)]$, where I_0 is the number of quanta of radiation entering the reaction vessel per square centimeter per second (correction having been made for reflection and absorption by the window of the cell), equation (25) may be written

$$+ d C_{AB}/dt = (A I_0/V) [1 - \exp(-a C_{A_2} l)] \frac{4 k_2 C_{B_2}}{k_4 C_M} \quad (27)$$

the quantum yield still being given by equation (26). If it is possible to expand the exponential and neglect the higher terms (either because the absorption coefficient is low, the concentration of A_2 low or l small), equation (27) becomes

$$+ d C_{AB}/dt = (A I_0 l/V) 4 a k_2 C_{A_2} C_{B_2}/k_4 C_M \quad (28)$$

and the reaction will apparently follow a simple second order equation. Since A , I_0 , l , V and a may be determined experimentally without difficulty, one may determine $(k_2/k_4 C_M)$. Determination of k_2 and k_4 individually can only be accomplished by some of the methods mentioned above. It must be emphasized that the rate in this case is proportional to the incident light intensity and that it makes little difference whether the light beam is homogeneous or whether it is strictly parallel. Equation (25) will be exact as long as the quantity $(I_a l A/V)$ is determined properly. With high concentrations, long path lengths and diverging beams the difficulties may be increased from an experimental standpoint, but the rate expression will still have some significance.

Let us now turn our attention to mechanism II. The situation here is definitely more complex. One finds

$$+ d C_{AB}/dt = 2 k_2 C_{B_2} (I_a/k_4)^{1/2} \quad (29)$$

Here however the rate refers to a particular volume element since the length of the chain is determined by the rate of reformation of A_2 and this in turn depends upon the square of the concentration of the atoms of A. In this case if the number of quanta absorbed per cubic centimeter per second varies from point to point, the over-all rate observed may not seem to follow any obvious relationship.

If Beer's law is assumed and the beam of radiation is strictly parallel and homogeneous, $I_t = I_0 \exp(-a C_{A_2} l)$ where I_t is the number of quanta transmitted per second per square centimeter at a distance l from the incident window of the cell. Then

$$d I_t/d l = -a C_{A_2} I_0 \exp(-a C_{A_2} l) \quad (30)$$

= decrease in number of quanta transmitted per sq. cm. per sec. in traversing a thickness $d l$ at a distance l from the incident window

= -number of quanta absorbed per cc. per sec. at a distance l from the window.

At a distance l from the incident window we may write

$$+ d C_{AB}/dt = 2 k_2 C_{B_2} (a I_0 C_{A_2}/k_4)^{1/2} \exp(-a C_{A_2} l/2) \quad (31)$$

This gives the instantaneous rate at only one point in the light path. The total number of molecules of AB formed per second will be

$$A \int_0^l (d C_{AB}/dt) d l = d N_{AB}/dt \quad (32)$$

and if the photochemical reaction is slow compared to diffusion of the end products one may write

$$(1/V) d N_{AB}/dt = (d C_{AB}/dt)_{av}. \quad (33)$$

$$(dC_{AB}/dt)_{av} = 4 A k_2 C_{B_2} (I_0/ak_4 C_{A_2})^{1/2} [1 - \exp(-aC_{A_2}l/2)]/V \quad (34)$$

In the simple case in which the exponential may be expanded in a series and higher terms neglected this becomes

$$(d C_{AB}/dt)_{av} = 2 A k_2 l C_{B_2} (I_0 a C_{A_2}/k_4)^{1/2}/V \quad (35)$$

This kind of a rate expression has been observed experimentally in several cases. The expression for the quantum yield is obtained by dividing both sides of the equation by the number of quanta absorbed per second, and multiplying by the volume.

$$\text{Quantum yield} = 2 C_{B_2} (1/I_0 k_4 a C_{A_2})^{1/2} \quad (36)$$

It is seen, therefore, that the yield is inversely proportional to the square root of the absorption coefficient. The importance of this statement is obvious if polychromatic radiation is used, since each wave length will have a different absorption coefficient. If $a = f(\lambda)$ and also $I_0 = f'(\lambda)$, the general expression for the rate of the reaction would become

No. molecules AB formed per second =

$$2 A k_2 C_{B_2} \int_0^l \left[\int_{\lambda_1}^{\lambda_2} f(\lambda) C_{A_2} f'(\lambda) \exp(-f(\lambda) C_{A_2} l) d\lambda \right]^{1/2} d l \quad (37)$$

If the source of radiation consists of a few discrete lines one of the integrations could be replaced by a summation.

Equation (34) gives the instantaneous rate for monochromatic radiation and dividing by

$$A I_0 [1 - \exp(-a C_{A_2} l)]$$

will give the instantaneous value of the quantum yield. However in an actual experiment the concentrations are allowed to vary by an appreciable amount in determining the quantum yield. Thus the actual number of molecules of AB formed divided by the total number of quanta absorbed over a given time interval will be the quantum yield. The resulting expression is very difficult to handle and little would be learned by pursuing this matter further. Suffice to say that in many experiments the differentials in equation (34) may be replaced by finite differences without introducing an appreciable error.

In this discussion we have considered only a beam of parallel radiation. For mechanism I it

is easily seen that the form of the rate equation will be independent of the geometry of the light beam. For mechanism II this is not so. Further we have treated the concentrations of the atoms A and B as though they were localized in the light beam, and the chains did not last long enough to make it necessary to take account of diffusion. Obviously all cases will be encountered experimentally from this idealized one to the other extreme in which the concentrations of the atoms may be considered to be uniform throughout the entire vessel. With the assumptions made in the above derivation a converging or diverging beam complicates matters enormously since in the narrow part of the beam the rate of recombination will be higher than it will be in the broad part of the beam.

For the reasons given in the preceding paragraphs one would scarcely expect any reaction which follows mechanism II to follow experimentally the ideal equation in which the rate is proportional to the square root of the light intensity and the first power of the concentration of B_2 . Deviations from the square root law may be due either to the difficulties connected with the light beam or the fact that the chains are terminated by a combination of the methods of mechanisms I and II. That these difficulties are of more than academic interest is evident from a perusal of recent work on photochemical halogenations. When sufficient care is observed in puri-

fying the reactants these reactions seem almost always to have rates proportional to the square root of the light intensity. And yet with the 3660 line of mercury as a source and chlorine pressures of even a few centimeters, the expansion of the exponential in equation (34) is hardly justified unless several terms in the series are included.

One further point may be mentioned in passing. Since in regions of continuous absorption molecules are supposed to be dissociated in one act, true quantum yields correctly determined would not be expected to vary much with wave length. At high pressures where the atoms produced can not separate very far before undergoing collision, recombination might be almost instantaneous and the number of reaction chains started be smaller than at low pressures. This has been advanced as an explanation of decreasing quantum yield of ozone formation with pressure⁸. Increase of frequency of the quantum absorbed would result in more rapid separation of the atoms and hence in starting more chains. At

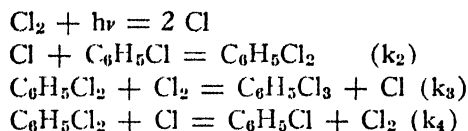
low pressures on the other hand the quantum yield for the primary process should be independent of wave length and the over-all quantum yield should follow equation (36) in which the yield is inversely proportional to the square root of the incident intensity multiplied by the absorption coefficient.

Little would be gained by discussing other hypothetical mechanisms in detail. One finds in the literature mechanisms of all shades and degrees of complexity. Many of these are justified by kinetic and other considerations and many are not. We will consider only one illustration of the difficulty of deciding upon a definite reaction mechanism from kinetic studies alone.

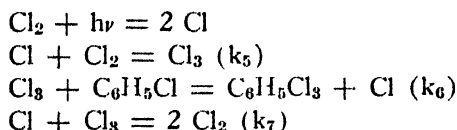
Rollefson and Eyring¹⁸ have suggested that chlorinations may proceed through the action of a triatomic chlorine molecule Cl_3 . While Sherman and Sun¹⁹ have concluded from theoretical considerations that this molecule is probably not important in addition reactions, Booher and Rollefson²⁰ feel that so many cases may be explained by its assumption that it should not be ignored.

Let us consider the following two possible mechanisms for the photochemical addition of chlorine to chlorobenzene²¹:

Mechanism III



Mechanism IV



By solving the quadratic equations for the concentrations of $\text{C}_6\text{H}_5\text{Cl}_2$ and Cl_3 and making similar assumptions with regard to the relative magnitudes of the various constants one obtains the following identical expressions for the rates:

For mechanism III

$$+ d (\text{C}_6\text{H}_5\text{Cl}_3)/dt = (I_a k_2 k_3 P_1 P_2 / k_4)^{1/2} \quad (38)$$

For mechanism IV

$$+ d (\text{C}_6\text{H}_5\text{Cl}_3)/dt = (I_a k_5 k_6 P_1 P_2 / k_7)^{1/2} \quad (39)$$

¹⁸ Rollefson, G. K. and Eyring, H., *J. Am. Chem. Soc.*, **54**, 170 (1932).

¹⁹ Sherman, A. and Sun, C. E., *ibid.*, **56**, 1096 (1934).

²⁰ Booher, J. E. and Rollefson, G. K., *ibid.*, **56**, 2293 (1934).

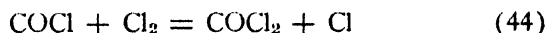
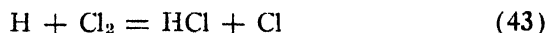
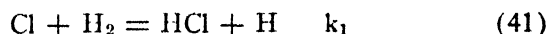
²¹ Cf. Hart, E. J. and Noyes, W. A., Jr., *ibid.*, **56**, 1305 (1934).

P_1 = chlorine pressure, P_2 = chlorobenzene pressure.

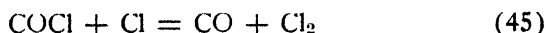
Obviously a study of the kinetics of the reaction alone will not decide between the two mechanisms. Unfortunately this situation is quite common in photochemical studies.

One method which may be used to determine the mechanism of a reaction, in case that of another is known, is to cause the two to take place simultaneously. If the light absorbing substance is the same in the two cases, then in general the chain stopping steps will be the same in the mixture. We will illustrate by means of the hydrogen chloride and phosgene syntheses. The chain stopping step in the phosgene synthesis is probably $\text{COCl} + \text{Cl} = \text{CO} + \text{Cl}_2$ ²², while in the hydrogen chloride synthesis the chains are generally assumed to stop on the walls (see mechanism I above) since the light intensity enters the rate equation to the first power. Rollefson²³ has caused these two reactions to take place simultaneously. While some of the conclusions have been questioned²⁴, the method is well illustrated by the study.

If a mixture of carbon monoxide, hydrogen and chlorine is illuminated by light absorbed only by the chlorine, the following reactions may take place



The chain stopping steps may be



The fraction of the chlorine reacting with the hydrogen may be shown to be

$$\frac{\Delta \text{H}_2 / (\Delta \text{H}_2 + \Delta \text{CO})}{1/[1 + k_2(\text{CO})(\text{Cl}_2)/k_1(\text{H}_2)]} = \quad (48)$$

where the parentheses indicate concentrations. If the active form of chlorine is Cl_3 , equations 41 and 42 become



²² Bodenstein, M., Lenher, S. and Wagner, Z. *phys. Chem.*, **B3**, 459 (1929).

²³ Rollefson, G. K., *J. Am. Chem. Soc.*, **56**, 579 (1934).

²⁴ See Bodenstein, M., Brenschede, W. and Schumacher, H. J., *Z. phys. Chem.*, **B28**, 81 (1935).

Instead of equation (48) we should have

$$\frac{\Delta H_2}{(\Delta H_2 + \Delta CO)} = \frac{1}{1 + k_5(CO)/k_4(H_2)} \quad (51)$$

In general if the active forms of chlorine are the same for both reactions equation (51) will result, while if they are different an equation of the type of (48) will result. In the phosgene synthesis it is immaterial whether one writes Cl + Cl_2 or Cl_3 . Rollefson²⁸ finds that if he plots the reciprocal of the fraction of the chlorine used by the hydrogen against the carbon monoxide pressure divided by the hydrogen pressure he obtains a straight line. From this he concludes that the active form of chlorine in the two reactions is the same. The further conclusion that Cl_3 is an intermediate in either reaction, is not justified, of course, from these considerations alone. The method is, however, a very useful one and has received other applications.

So far we have said very little about the dependence on temperature of photochemical reactions. In case a molecule is dissociated by light and the subsequent reactions involve only a recombination of fragments without continuing chains, the quantum yield will probably not vary greatly with temperature. Due account must be taken of the variation of absorption coefficient with temperature, however. Many photochemical reactions are known in which the increase in rate amounts to only a few percent for each ten degree rise in temperature.

One of the general characteristics of a continuing chain reaction²⁵ is that there will exist a temperature at which the reaction velocity will not follow the simple Arrhenius equation $d \ln k/dT = E/RT^2$. This is ascribed to the fact that the length of chain varies with the temperature and that the number of chains initiated will also vary with temperature.

The rates of photochemical chain reactions may either increase or decrease with temperature. To illustrate let us return to mechanism I above and replace each of the velocity constants by the expression obtained from the Arrhenius equation

$$k_1 = s_1 \exp(-E_1/RT) \quad (52)$$

If the reaction is of such a nature that the assumption of the steady state is justified over the temperature range investigated, equation (28) may now be written

$$+dC_{AB}/dt = 4(AI_0/VC_M)a[s_2 \exp(-E_2/RT)/s_4 \exp(-E_4/RT)]C_{A_2}C_{B_2} \quad (53)$$

If the constant were being determined as though the reaction were second order, the constant k for the over-all reaction would be

$$k = (4AI_0/V)(s_2/s_4)a \exp[-(E_2-E_4)/RT] \quad (54)$$

and differentiating logarithmically to put the equation in the Arrhenius form we find

$$d \ln k/dT = d \ln a/dT + (E_2-E_4)/RT^2 \quad (55)$$

If the absorption coefficient a is not independent of the temperature the reaction may appear to be either Arrhenius or Anti-Arrhenius. Since there is no *a priori* reason why (E_2-E_4) should be either positive or negative one will expect to find both positive and negative variations of rate with temperature. If the chain stopping process, equation (23), involves removal by the walls E_4 will be small or zero and the expected behavior would be an increase of rate with temperature.

In more complex reactions one will expect to find the behavior more complex and as already stated²⁵ the sign of the apparent energy of activation for the over-all reaction may change as the temperature increases. In some cases, of course, the branching of chains may become increasingly important with rise in temperature or change in other experimental conditions and a sufficiently exothermic reaction may become explosive.

In this hasty survey of the subject of chain reactions no attempt has been made to give a complete or comprehensive bibliography of the subject. The examples chosen have been partly hypothetical and partly real, and any other worker in the field may take exception to the choices made. Large parts of the subject have been left untouched, but it is hoped that some of the important practical aspects of the subject will have been treated so that the difficulties in this type of study will be apparent. It can not be emphasized too strongly that this field has been strewn with wasted effort because the errors which one may make in proving a reaction mechanism have not been clearly understood. In relatively few cases in the literature does one find complete and convincing proof of one mechanism for a given reaction as distinguished from others.

But one must not take too pessimistic a view of the subject. The number of carefully studied reactions is increasing rapidly now that improved experimental technique is available and a clearer realization of the theoretical pitfalls has become more general. Nevertheless in many cases one must be content to do the best one can with the apparatus and the mentality available, realizing full well that the ideal has not been reached.

²⁵ Cf. Taylor, H. S. and Salley, D. J., *J. Am. Chem. Soc.*, **55**, 96 (1933).

DISCUSSION

Dr. Kassel: I have two comments to make. The first deals with the construction of chain mechanisms in the case that the chains are very long and that no plausible chain-carrying steps involve reaction between two or more chain carriers. Then one can write all possible chain reactions, calculate the most general rate expression, and see what particular choices of constants will make this reduce to the experimental expression. It will in many cases be found that several different choices are satisfactory, but by such a treatment there is reasonable assurance that no possibility has been overlooked.

The second comment is that when the steady state assumption is not valid, the reaction usually cannot be followed by pressure changes alone, since the intermediate will be present in appreciable quantity. Then such an intermediate can be determined analytically and the steady state assumption need not be applied to it.

Dr. Bates: I should like to question Noyes' definition of a chain reaction as consisting of any reaction which occurs through the intermediate formation of atoms or radicals. I had thought that the term was usually applied to reactions in which one of the intermediates was regenerated in the course of the reaction. For example, I should call the hydrogen-chlorine reaction a chain reaction, but would not use the term to describe the photodecomposition of hydrogen iodide, although atoms appear in the mechanism of the latter reaction.

Dr. Noyes: I have never had any one agree with my definition. When I tried to restrict it, as you have suggested, it seemed to me the distinction between chain and non-chain reactions became vague.

Dr. Rollefson: The difficulty in deciding between various possible steps in a chain mechanism on the basis of calculations such as Eyring makes, lies in the fact that in those calculations the error is sometimes 10 large calories or greater, whereas very often the heats of activation for the steps in the chain process are not more than 10 large calories. Take one particular reaction which Eyring has discussed, namely whether to use Cl or Cl₂ in the hydrogen chloride synthesis. He calculates the heat of activation for chlorine atom plus hydrogen molecule to be 16 large calories and for Cl₂ plus hydrogen about 21. Now from an experimental standpoint Cl plus H₂ has a heat of activation of 6 large calories. Thus there is a discrepancy between theory and experiment amounting to at least 10 large calories and yet the theory is trying to decide between two reactions for which the calculated difference is only 5 large calories. That is why I still use triatomic halogens when I find any need for them.

Dr. Kassel: My feeling, based on less specific methods than those Eyring uses, is that the triatomic halogens will, however, in all cases probably have a slightly higher activation energy than the free atoms and that only in dealing with association reactions where having the Cl₂ as the second body in the reaction product is helpful, will the reaction by way of the triatomic form be favored.

Dr. Rollefson: At present that is the only type of reaction for which it is necessary to bring in that assumption.

Dr. Noyes: There is one further point I might have mentioned and that is the method of competing reactions. I would like to hear Rollefson comment on the CO—Cl₂ and H₂—Cl₂ reactions, with particular reference to the recent work from the Bodenstein laboratory.

Dr. Rollefson: In the particular example cited by Noyes, there are some discrepancies between the results obtained in Bodenstein's laboratory and my experiments. This is at least partially due to differences in procedure, as in our experiments the percentage of the substances reacting was kept as low as possible in order to avoid complications due to the reaction products, whereas the experiments performed in Bodenstein's laboratory sometimes involved as much as ninety percent reaction. As they report disturbances due to the reaction products, the two sets of results cannot be compared quantitatively. At present I feel that the only conclusion which can be drawn from these experiments is that the active form of chlorine is the same in both the phosgene and hydrogen chloride syntheses, with chlorine having an additional effect in the phosgene reaction. I believe that COCl is definitely an intermediate, although Bodenstein discussed his results on the assumption that I had discarded that intermediate.

Dr. Bates: In this connection, would it not be possible to determine whether Cl₃ is formed by measuring the absorption spectrum of strongly illuminated chlorine in the far ultraviolet, as has been done by Turner? By varying the chlorine pressure the absorption due to chlorine atoms should vary according to the equilibrium forming Cl₃ from chlorine atoms.

Dr. Rollefson: That would be a very difficult experiment, because if the chlorine pressure is increased there would be an increase in the number of chlorine atoms formed due to increased absorption and at the same time the higher pressure of chlorine would convert more Cl into Cl₂. These processes have opposite effects on the Cl concentration and therefore it would be necessary to detect very small changes in this concentration.

I believe that the best chance of detecting the triatomic halogens lies in a study of the vapor

densities of bromine and iodine at high temperatures. At present there is a discrepancy between the theoretical equation for the dissociation of iodine and that for bromine (both based on spectroscopic data) and the experimental values which have been obtained by Bodenstein. For iodine the discrepancy amounts to about 10 percent of the equilibrium constant at 1000° C. and for bromine it is 30 percent. This discrepancy can be eliminated by assuming the presence of triatomic halogens with reasonable assumptions as to their stability. At present the experimental data are not sufficiently complete to test the variation of the discrepancy with temperature and pressure in order to see if these will fit the form required by the assumption of triatomic halogen molecules.

Dr. Noyes: Concerning Bates' point, I think one would have to look for a line absorption due to chlorine atoms at wavelengths where the difficulties would be great due to absorption by chlorine molecules.

Dr. Hartline: I do not wish to get the discussion off the track, but I might remark that this is getting very close to certain problems in biology concerned with the visual mechanism. For example, it is necessary to assume some sort of steady state, if for no other reason than that one can stay in lighted surroundings without going blind. Moreover the sensitivity after one has become completely accustomed to a given light intensity remains constant. I presume that this discussion largely referred to gaseous photochemistry, but I wonder whether any liquid systems exist, or possibly could be found, involving chains. I think a study of these chain reactions would be very valuable for biologists.

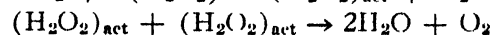
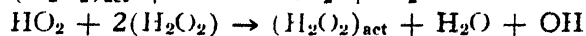
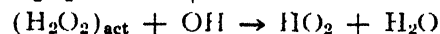
Dr. Noyes: Of course chain reactions in liquid systems are known: one is the chlorination of tetrachloroethylene in carbon tetrachloride solution. I do not know, off hand, of any aqueous system—I presume they must exist.

Dr. Forbes: The oxidation by air of sulphites.

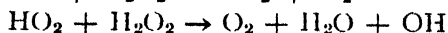
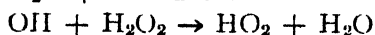
Dr. Noyes: The assumption I made in deriving these expressions is to the effect that all reactions involving intermediates take place in the light path. One must have variations from this situation. Of course, if atoms or radicals of long life diffuse out of the light path, the treatment of the problems becomes more difficult and the shape of the vessel may have to be considered. In general we know little concerning diffusion processes in liquids.

Dr. Fricke: Discussing exothermic reactions of the type for which the quantum yield varies with the light intensity, Noyes pointed out the experimental difficulty of obtaining a uniform intensity throughout the irradiated volume. For this reason, in some cases more readily interpretable results are obtained when X-rays are used.

For example, we have studied the decomposition of solutions of hydrogen peroxide by this agency and find the decomposition, for equal units of X-ray energy absorbed, increases as the square root of the hydrogen peroxide concentration and inversely as the square root of the X-ray intensity. The study of this reaction with light, as carried out by a number of different investigators, has not so far given completely satisfactory results. However, the variation of the quantum efficiency as the square root of the concentration and as the inverse square root of the light intensity gives the best summation of all the data. We have seen no wholly satisfactory way to explain these relationships, but the following equations may be set down as perhaps containing something of the true mechanism:



Dr. Kistiakowsky: There is fairly good evidence that in ultraviolet light hydrogen peroxide gives hydroxyl radicals. The work of Urey, Dawsey and Rice has established that upon illumination of hydrogen peroxide vapor with light of sufficiently short wavelength, fluorescence of hydroxyls is observed. Evidently one obtains one normal and one excited hydroxyl radical. Of course, I am not sure that X-rays will produce the same products as light, but are not the kinetics of the X-ray reaction very similar to the kinetics of the photochemical reaction and should not one conclude from this that the primary process is the same? If I may write the Haber and Willstätter mechanism:



Now you have the continuation of the chain, and it seems to me that if you assume that the chain is ended by recombination of radicals, you get the correct relation of rate and radiation intensity.

Dr. Kassel: I may be wrong, but I think the two mechanisms will lead to very similar results.

Dr. Fricke: The fact that the decomposition depends on the X-ray intensity shows that the rate of decomposition must be regulated by collisions between two activated molecules. This must be the case whatever mechanism we assume. The number of hydroxyl radicals in equilibrium with hydrogen peroxide of a certain concentration depends upon the heat of dissociation, which is not well known. The value 30 Cal. seems a probable one. If this is the correct value, the concentration of OH would not seem to be too

low to make the proposed mechanism an unlikely one.

Dr. Bates: Fricke's mechanism calls for the collision of an activated hydrogen peroxide molecule with a hydroxyl which is in its equilibrium concentration. This concentration would seem to be so low as to preclude the possibility of such a process, when it is remembered that the activated hydrogen peroxide would have to make many collisions with other molecules before meeting one of the few hydroxyl groups.

Dr. Brackett: There is one comment I would like to make. It does not bear on matters of mechanism, but has to do with the fact that in biology one is frequently concerned with wavelengths shorter than 3000 Å. In that region Eller has found some evidence that ionization does occur. For instance, in normal saturated hydrocarbons, when illuminated with various wavelengths, a conductivity can be measured with a threshold which depends on the size of the hydrocarbon molecule. The threshold shifts toward longer wavelengths with increasing size and also for branched hydrocarbons. For complicated molecules this may occur at wavelengths even as long as 2500 to 2900 Å. Much biological photochemical work has been done in this region and while it may be surprising to find ionization at this point, without knowing more about the liquid ionization potentials it is impossible to say whether ionization would be of importance or not.

Dr. Noyes: Certainly many reactions may be initiated by ionization. In most photochemical reactions I do not think that chain mechanisms involving ions are necessary.

Dr. Bates: I should think it hardly possible for electrons to be removed from hydrocarbons as a primary act of the absorption of light of wavelength 2500 Å. The electrons are more tightly bound than in metal lattices. Some of the originally formed radicals might react on the surface of the metallic electrode, giving rise to what might be called a "chemi-electric" effect and producing electrons. This is, of course, mere speculation.

Dr. Brackett: No. We have only the over-all observation that they are formed and that conductivity follows, or rather loss of conductivity follows very slowly subsequent to illumination, a matter of many minutes.

Dr. Bates: There are cases, I believe, in which collisions of the second kind between molecules and electrons may produce electrons of high velocity. Radicals may react on the surface of the electrons which are present, thus causing electrons to be ejected from the surface.

Dr. Brackett: We attempted to exclude that possibility by having the electrodes shielded and at some distance from the illuminated portion of the material. That was as much as we were able to do.

Dr. Bates: I was objecting to the appearance of ions in the primary act.

Dr. Brackett: I think it unlikely that they arose from the electrodes rather than in the body of the materials under those conditions. The long distance that activated molecules would have to travel would make it rather surprising to me that they would reach the electrodes.

Dr. Kassel: In almost any reaction one finds the production of a few electrons, one per million or ten million. Electrons seem to have very long lives in liquid hydrocarbons. Might not gradual accumulation of these few electrons be more plausible than direct ionization with an energy of only four volts?

Dr. Brackett: Until the value of the ionization potential in liquid as compared to gas is better known, it seems difficult to arrive at an adequate basis for judging as to the plausibility of an ionization potential range of from four to five volts in liquid.

Dr. Bates: Not very difficult. In metals the electron is pretty loosely attached and gives rise to metallic conduction. In hydrocarbons this is not so. The energy here necessary to remove the electron would be fairly close to the ionization potential of the gas.

PHOTOCHEMICAL FORMATION AND REACTIONS OF RADICALS AND ATOMS

G. B. KISTIAKOWSKY

It has been recognized for some time that in photochemical reactions two stages are readily distinguished: the primary and the secondary processes. The first comprises the process of light absorption and the resultant changes in the absorbing system (an atom or a molecule), while the second includes all subsequent reactions of the active species produced with other atoms or molecules present. The primary process is mainly determined by the size of the light quanta and the nature of the light absorbing particles, although in some instances it may be altered by the presence of other molecules. The secondary reactions, on the other hand, are determined by the composition of the system studied and are in no essential detail different from reactions caused by such active particles when these are produced thermally or by means of electric discharges. Superficially, however, differences may be observed because the use of photochemical methods enables one to study reactions under such conditions of temperature and pressure that no other methods can be applied and thus some reactions (those with low activation energy or reactions requiring triple collisions) are enhanced at the expense of others.

A production of atoms or free radicals requires a rupture of chemical bonds in the light-absorbing molecule and unless these linkages are weak (as in halogen compounds) *large* amounts of energy are required for the process. In agreement with this is the general observation that most photochemical reactions involving atoms and radicals are caused by ultra-violet light, except with halogens and one or two other unstable compounds.

Spectroscopic studies have gradually shown that all molecular spectra of vapors and gases may be subdivided into three main classes: banded spectra with discrete rotational structure; diffuse bands; and continua. In liquids and solids, except at very low temperatures, only diffuse spectra are known and this was early attributed to the mutual interaction of the closely packed molecules. In gases, on the other hand, each of the above types of spectra indicates a different primary process.

The discrete spectra are taken as evidence that upon absorption of light a stable activated molecule is formed which, after an average interval of something like 10^{-8} seconds, emits (in one or several steps) the absorbed light energy as fluorescence, unless the intervening molecular collisions alter this. Examples of this type of spectra are the halogens in the red and yellow regions of the visible spectrum, nitrogen dioxide in the visible and formaldehyde or benzene near the beginning of their ultraviolet spectra.

The diffuse bands (the degree of diffuseness varying from molecule to molecule and from one part of the spectrum to another) have been interpreted as indicating "predissociation":^{1, 2} the activated molecule is assumed to undergo an electronic rearrangement without emission of light by which it becomes unstable and immediately decomposes. For this process to produce a diffuse spectrum, it must occur much more rapidly than the ordinary process of fluorescence and times varying from 10^{-10} to 10^{-12} seconds can be estimated for it. In order that predissociation may occur, two electronic levels must have an identical energy at the same spacial configuration of the atomic nuclei in the molecule and also must fulfill other requirements. Thus predissociation, while widespread, is not universal and even with those molecules with which it has been observed at all, it is usually limited to a part of the absorption spectrum, being preceded from the red by discrete bands and followed to the violet either by a continuum or, frequently, by other discrete bands. Examples of predissociation are particularly numerous with polyatomic molecules: ammonia and ozone have only diffuse bands; formaldehyde, other aldehydes and ketones have predissociated bands in the middle ultraviolet, as have benzene and its derivatives below 2200-2500Å. Some diatomic molecules (S_2 , AlH) are also known to have diffuse bands.

The continua in molecular spectra are taken to be the evidence that the excited state of the molecule is itself unstable³, so that upon absorption of light the molecule dissociates instantaneously. Halogens in the blue and violet of the visible and in the near ultraviolet, the oxygen molecule below 1750Å, nitrosyl chloride in the visible, hydrogen peroxide in the ultraviolet and probably many organic molecules in the far ultraviolet (2500-2000Å) exhibit this type of spectra.

Such an interpretation of molecular spectra leads to the conclusion that a formation of atoms or radicals should be expected in all* cases when continuous spectra have been established for substances studied as dilute gases. This is indeed true experimentally. The halogens and the halogen hydrides are the classical examples of atom production with light of wave lengths belonging to their continua; oxygen atoms are known to be formed when oxygen molecules are illuminated by light below 1750Å. Among polyatomic molecules, the case of hydrogen peroxide is particularly clear⁴. Two hydroxyl radicals are formed

* Except when the molecules decompose to form other stable molecules.

here and if the light is of sufficiently short wave length, one of the radicals is formed in the excited state. This is shown by the emission of hydroxyl bands as fluorescence. Many other similar reactions are also known. It has been uniformly assumed in these cases—and no evidence to the contrary is available—that every molecule absorbing light decomposes, so that the quantum yield is constant and is equal to unity, for the primary reaction at least.

Predissociation spectra are also normally expected to yield decomposition products, but since the time between absorption of light and decomposition is here appreciable and may be even longer than the average time between molecular collisions at moderate gas pressures, it is conceivable that the activated molecule may undergo some other reaction on collision instead of decomposition, or even lose its energy in this manner. These considerations apply, of course, particularly to reactions taking place in condensed phases.

Some of the better known reactions involving predissociation are the decomposition of ammonia, which yields NH_2 and a hydrogen atom, the decomposition of ozone (both in light of the red and the ultraviolet regions of absorption), yielding an atom and a molecule of oxygen⁵, and decompositions of nitrogen dioxide⁶, of aldehydes and of ketones. In several such reactions the quantum yield has been observed to decrease with increasing wave length near the lower limit of the predissociation spectrum. This behaviour may possibly be accounted for by assuming that the probability of predissociation decreases and thus the molecules have an increasing chance to lose beforehand their energy on collisions. A case of this type is the decomposition of ketene⁷ and others will be mentioned. In liquid systems, as stated before, the time between impacts is so small that the fate of activated molecules will, to a very great extent, be determined by their behaviour in collisions. These, as pointed out, may lead to a loss of energy, but also may lead to reaction. In any event reactions in which even stable activated molecules undergo decomposition are known. Thus the kinetics of the hydrogen bromine reaction are unchanged when light is used of wave lengths longer than the limit of the bromine continuum⁸; this clearly indicates that free bromine atoms are formed on collisions of activated bromine molecules with other molecules present. In the case of oxygen, illuminated by light of longer wave lengths than 1750\AA , atoms are formed only as the result of a secondary reaction of activated molecules⁹: $\text{O}_2^* + \text{O}_2 \rightarrow \text{O}_3 + \text{O}$. These last two reactions may serve as examples of atom formation when spectra are discrete, but others are known.

Besides the direct formation of atoms or radicals, the process of sensitized decomposition must be considered, of which again many examples could be quoted. The most frequently investigated reactions of this type are those caused by excited mercury atoms, several of which are known. They involve decomposition of hydrogen, ammonia, hydrocarbons and some others. The exact mechanism of the majority of these reactions is unknown as yet. In the case of hydrogen it is now assumed that a secondary reaction of the excited mercury atom, rather than an energy transfer, leads to the atom formation¹⁰, $\text{Hg}^* + \text{H}_2 \rightarrow \text{HgH} + \text{H}$, and the same may be true of the other processes mentioned.

Space will not permit even a brief mention of all reactions which the photochemically formed atoms or radicals are known to undergo. Instead, only a few cases will be selected arbitrarily and discussed in some detail. Such a treatment must inevitably include a consideration of other evidence gathered on these active species, besides the photochemical information, because frequently corroborative or complementary data are thus obtained. In the case of hydrogen atoms, for instance, much of the evidence has been gathered from experiments with atoms withdrawn from electric discharges at low pressures¹¹. The data agree in general with what is known from the study of hydrogen atoms produced by excited mercury or by decomposing ammonia, although some discrepancies are noted. Some of these are, in all probability, real and are to be attributed to the large difference of pressures used in the two types of investigation, a condition which favors different reactions when such are chemically possible.

The reactivity of hydrogen atoms in general has been found to be very high, although their exchange reactions with stable molecules seem to require some activation energy. Thus the exchange with hydrogen molecules¹², $\text{H} + \text{H}_2 \rightarrow \text{H}_2 + \text{H}$, involves about 7 Cal., while the exchange with methane¹³ (studied by means of deuterium atoms), $\text{D} + \text{CH}_4 \rightarrow \text{CH}_3\text{D} + \text{H}$, takes about 5 Cal. No exchange reaction of the type $\text{H} + \text{CH}_4 \rightarrow \text{CH}_3 + \text{H}_2$ has been observed even at 150°C .¹¹ and must therefore require quite a large activation energy. With other saturated hydrocarbons, however, this reaction proceeds quite rapidly and also the reaction involving a rupture of the carbon to carbon bond, judging from the reaction products, must be taking place¹¹. It appears then that methane occupies a somewhat exceptional position and it would be of great interest to determine whether this is due to a particularly strong binding of the first hydrogen on methane or to other causes. It may be noted that also ammonia is quite stable against hydrogen atoms¹¹, while hydrazine undergoes a

rapid reaction¹⁴. The case is perhaps not quite analogous to that of hydrocarbons since with hydrazine the entire reaction may involve only the nitrogen to nitrogen bond. With oxygen and carbon monoxide hydrogen atoms undergo addition reactions which are apparently triple collision processes and do not require activation energy of any appreciable amount^{15, 16}. The radical HO₂, through a sequence of reactions which is known but uncertainly, forms mainly hydrogen peroxide; the radical HCO forms formaldehyde and glyoxal in varying proportions.

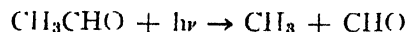
It could be expected that the reaction of oxygen atoms with hydrogen molecules is similar to that of hydrogen atoms and oxygen molecules, thus leading to the formation of water in a triple collision process. Instead, this reaction, while requiring triple collisions, results in about equal amounts of hydrogen peroxide and water⁹ and must be thus more complex than $O + H_2 + M \rightarrow OH_2 + M$. At low pressures, apparently, another reaction sets in, $O + H_2 \rightarrow OH + H$, which requires about 6-7 Cal. activation energy¹⁷.

Our knowledge of the reactions of hydrogen and oxygen atoms is inferior to that of the reactions of bromine and chlorine atoms, of which many are known. Without delving into them any more, however, we shall turn our attention to reactions of free radicals, in particular to processes occurring in decomposition of aldehydes and ketones. The majority of these form carbon monoxide and a hydrocarbon as the result of illumination. None of the reactions is entirely clean, however, and other products, as hydrogen in the case of aldehydes¹⁸, are usually observed, while in a few instances the reaction takes an altogether different course. The quantum yields at room temperature are usually small, less than unity. That of acetaldehyde is of the order of unity at 2536Å and about 0.3 at 3130Å¹⁸; propionaldehyde yields are slightly higher but vary in the same manner¹⁹; the quantum yield of acetone is about 0.2-0.3 and depends somewhat on the light intensity²⁰; ketene has a yield of about 0.2 at 3600Å and about 1.0 at 3100Å⁷. Leermakers has recently shown that these quantum yields are a function of temperature and rise to several hundreds at 300-400° C. when acetaldehyde is used²¹. With acetone they rise to approximately unity and then remain fairly constant from about 180 to 400° C.²² As Leermakers points out, these observations force one to the conclusion that a primary reaction must be followed here by secondary processes which, under favorable conditions, can form long chains.

Much of our knowledge of the decomposition of aldehydes and ketones is due to the work of Norrish²³. From a study of the reaction products at room temperature he has arrived at the con-

clusion that the mechanism of the primary decomposition of aldehydes is the splitting of a CO group, a stable hydrocarbon being also formed. The deficiency of the quantum yields is accounted for by assuming that an *internal* dissipation of energy from the reacting bond takes place very soon after predissociation and before the molecule has a chance to decompose. This is regarded as the reversal of the process which is responsible for the thermal unimolecular reactions. The more energy the molecule has absorbed, the more likely it is that enough will remain in the reacting bond to cause decomposition. Thus the quantum yield should be increased by decreasing the wave length of radiation as actually found. This explanation is entirely acceptable, but a reconsideration of the question of the deficient quantum yields from the point of view of the reaction chains, in particular of their dependence on light intensity, must be made before the final decision is reached.

A primary formation of carbon monoxide and of stable hydrocarbon, as suggested by Norrish, seems to be out of the question, because of the observations of Leermakers at higher temperatures. The latter assumes, in accordance with several earlier suggestions¹⁸, that the primary reaction is of the type



and is followed, when the temperature is sufficiently high, by a variety of reactions of the methyl radicals. The formyl radical, under these conditions, decomposes, yielding CO, while the hydrogen atom formed also undergoes several reactions. The decomposition of the formyl radical occurs not instantaneously upon its formation and presumably the process requires some activation energy obtained from collisions. Not only this, but also other reactions occurring in the chain require activation energy and thus with decreasing temperature the chain is retarded. At room temperature the main reactions are the reactions of the free radicals among themselves, since these require the least activation energy. The predominant reaction is $CH_3 + CHO \rightarrow CH_4 + CO$, but possibly the recombination $CH_3 + CHO \rightarrow CH_3CHO$ is also taking place and accounts for the deficient quantum yield. The small amounts of hydrogen found among the decomposition products at lower temperatures are due to the decomposition of the formyl radicals and subsequent recombinations of hydrogen atoms or their reactions with aldehyde molecules. That no ethane apparently is found among the decomposition products of acetaldehyde is entirely in accord with observations of Rice²⁴ on reactions of free methyl radicals, who also finds that such reaction is very slow.

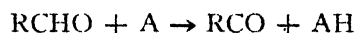
By means of radicals here discussed, Leermakers is able to derive kinetic expressions for the rate of photochemical decomposition which are in accord with his experimental observations and agree also with Rice's interpretation of the thermal decomposition of acetaldehyde and other organic compounds²⁴.

For the decomposition of lower ketones Norrish suggests a primary process consisting in a simultaneous severance of both hydrocarbon radicals from the CO group. The radicals then recombine and thus, if an unsymmetrical ketone (methylethyl) is studied, all three possible hydrocarbons are found. Further support for this view is claimed from observations on the decomposition of cyclic ketones, which yield mainly cyclic hydrocarbons and also from the finding of Pearson²⁵ that free methyl radicals can be observed in decomposing ketones but not in acetaldehyde.

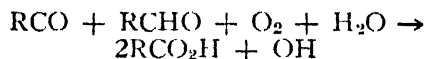
Contrary to this view, Leermakers assumes that only one radical is severed from the original molecule. At elevated temperatures, when the quantum yield is nearly unity, the residue of the acetone molecule (OCCH_3) decomposes unimolecularly, whereupon a recombination of the free methyl (and ethyl) radicals takes place, since other reactions would require too much activation energy. At lower temperatures the radical OCCCH_3 is supposed to be sufficiently stable to undergo mainly the recombination reaction $\text{OCCCH}_3 + \text{CH}_3 \rightarrow \text{CH}_3\text{OCCH}_3$, accounting for the low quantum yield. A reaction of the type $\text{CH}_3 + \text{CH}_3\text{COCH}_3 \rightarrow \text{CH}_4 + \text{CH}_2\text{COCH}_3$ must also be taking place to some extent since methane is found in appreciable quantities²⁰. Evidence adduced by Norrish in support of his theory serves equally well for this mechanism. Thus the difference with respect to free radicals found by Pearson between acetaldehyde and ketones is explained by their rapid exchange reaction in the first case and longer mean life due to the slow recombination in the second. The only possible difficulty is presented by the case of cyclic ketones. It is necessary to assume that the recombination of radicals like $\text{C}_6\text{H}_{12}\text{CO}$ is too slow to take place to any appreciable extent and that therefore they decompose by $\text{C}_6\text{H}_{12}\text{CO} \rightarrow \text{C}_6\text{H}_{12} + \text{CO}$, forming a hydrocarbon radical with free valences on both ends of the chain. These, again, are not stable and either rearrange (into rings when the chain is long enough or otherwise into olefinic hydrocarbons) or break up into smaller molecules of olefinic hydrocarbons. The idea of instability of long-chain free radicals is quite in agreement with the views of Rice²⁴. In support of Leermakers' mechanism can be adduced also the observation of Damon and Daniels²⁰ that the quantum yield of acetone decomposition decreases with increasing light intensity. This is due, of course, to the limited life of the

OCCH_3 radical, favoring the formation of CO and ethane at low light intensities. It becomes also unnecessary to assume that with longer chain aliphatic ketones the primary process is different from the above and involves a rupture of one carbon-to-carbon bond in the middle of the hydrocarbon chain as assumed by Norrish. Instead we postulate, as before, that the rupture occurs at the carbonyl carbon, but that the long-chain radical is unstable and decomposes into a methyl and an olefin. The former then reacts, as in the case of acetone, with the OCCH_3 radical to form acetone which experimentally is one of the main reaction products. According to this development of Leermakers' idea, several crucial tests can be devised to prove its correctness. Thus the decomposition of methylbutyl ketone should give no acetone at somewhat elevated temperatures (150–200° C.) where the quantum yield of acetone decomposition approaches unity, since the OCCH_3 radical is unstable then. Also, in the decomposition of methylethyl ketone some acetone and some diethyl ketone should be found among reaction products.

In an intimate relation to the mechanism of aldehyde decomposition stands the problem of their photochemical oxidation in the condensed phase, both undoubtedly being started by the same primary process. But also the mechanism of their thermal oxidation (auto-oxidation) must be closely related, since, as Baekström²⁶ has shown, the photochemical and the thermal reactions are very much alike. The oxidations, as shown by Baekström, are chain reactions and it is clear in view of the preceding that free radicals must be involved in them. Radical mechanisms have indeed been proposed, one by Haber and Willstaetter²⁷, another by Baekström²⁸. In some respects these mechanisms are different from one another and also from the mechanism discussed above. According to Haber and Willstaetter the primary process in a thermal oxidation induced by a catalyst (A) is as follows:



and, accordingly, in a photochemical reaction: $\text{RCHO} + h\nu \rightarrow \text{RCO} + \text{H}$. This is followed by reactions which can be written summarily as:



the hydroxyl radical taking up the chain and reacting with another aldehyde molecule to form the acyl radical. The authors feel that this type of reaction is quite general and accounts for many organic oxidations and, in the absence of free oxygen, for disproportionation reactions.

Baekström's mechanism differs from the preceding one in that, following an idea of Boden-

stein developed to account for the thermal oxidation of acetaldehyde in the vapor phase²⁰, the primary process is supposed to be the loosening of the carbonyl linkage, a radical with free valences on carbon and on oxygen resulting. This then, in a reaction with aldehyde molecules, produces other radicals which carry on the chain by forming peracid upon reaction with oxygen. No free hydroxyls are supposed to be formed. Such a view of the reaction mechanism must be declined, however, because it does not conform with the evidence of spectra. The loosening (or partial breaking, if preferred) of the carbonyl bond represents, after all, nothing but another electronic state of the molecule and therefore discrete spectra should result. This not being the case, the mechanism of Baekström must be rejected.

The Haber-Willstaetter mechanism, on the other hand, is not subject to this criticism and, as a matter of fact, it will account equally well for the observations of Leermakers at elevated temperatures, as the mechanism proposed by that author. Its main difficulty is that at room temperature large amounts of hydrogen should result from the photochemical decomposition of aldehydes, which is not the case. The question of the primary formation of hydrogen atoms in these reactions could perhaps be decided by a study of the photochemical decomposition in the presence of para-hydrogen or deuterium, in which case the hydrogen atoms would show themselves through their exchange reactions with hydrogen molecules. In the meantime one is faced with the dilemma of either accepting the formation of hydrogen atoms and somehow explaining the absence of hydrogen in photochemical decomposition, but on the other hand being able to organize a vast amount of experimental observations from very different sources under a uniform point of view; or of asserting that the mechanisms of the photochemical and of thermal oxidation of aldehydes are different. This follows because methyl radicals seem to be unlikely carriers of chains in oxidation reactions at room temperature. The problem, as has been seen from this brief review, has many ramifications and its speedy solution is therefore very desirable.

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DISCUSSION

Dr. Kassel: To start with, I have a small pebble to throw, but I don't know whether it is aimed at Kistiakowsky or at Rice. The historical reason that Rice became interested in free radicals was that he found it hard to believe in such a reaction as the direct formation of H_2 and C_2H_4 from C_2H_6 . He thinks it is too complex a process to occur in one step, since two hydrogen atoms must split from different carbon atoms and become bound to each other. He likes to write reactions which involve the break of only one bond without rearrangement. Hence he thinks that $CH_3CH_2CH_2CH_3$ decomposes to $C_2H_5 + C_2H_4$, and $CH_3CHCH_2CH_3$ to $CH_3 + C_3H_6$. Thus to get C_3H_6 from methyl *n*-butyl ketones, you have to violate Rice's rules.

Dr. Kistiakowsky: I am willing to do that, since the reasoning of Rice which you outlined does not appear very convincing to me.

Dr. Kassel: As I say, I don't know where the pebble was aimed.

Dr. Bates: Thermal oxidation of acetaldehyde in the vapor phase takes place at 60°. Can one break the bond at 60° and obtain free radicals in sufficient numbers?

Dr. Kistiakowsky: Yes, if the chains are long enough. However, I have not meant to give the impression that in oxidation the same radicals carry the chain as in simple decompositions, I only meant that it is quite possible that the primary step is the same, although the mechanism of oxidation is different in details from the mechanism of decomposition.

Dr. Bates: Your oxidation mechanism is really a peroxide mechanism. It is, of course, identical with the mechanism of the oxidation of the free methyl groups. Here a peroxide is formed and another methyl iodide collides with the peroxide to give alcohol plus aldehyde.

Dr. Kistiakowsky: I have not thought of that connection. You are quite right, it is the same mechanism.

Dr. Rollefson: In this mechanism, how do you account for the variation in the amount of hydrogen produced as the wavelength is varied?

Dr. Kistiakowsky: I first heard of it from the letter of Leighton. It seems to me that it is not at all difficult. It is only reasonable to assume that with decreasing wavelength of absorbed light the kinetic energy, with which the two parts of the aldehyde molecule fly apart, increases. As the energy increases, the chance of COH to be decomposed increases also.

Dr. Rollefson: Another way of having hydrogen formed in these decompositions is to have two HCO radicals react with each other to give hydrogen and carbon monoxide. If the rate of this reaction is assumed to be slower than the rate of the reaction between the alkyl groups and HCO, then only a small amount of hydrogen would be formed. As the wavelength of the light is decreased, the HCO radicals formed would have more vibrational energy which might increase the rate of reaction between two HCO groups and thus increase the amount of hydrogen formed.

Dr. Bates: Such a mechanism does not agree with the evidence obtained from the mercury sensitized formation of formaldehyde. The HCO groups formed in this reaction undergo an addition reaction to form glyoxal and the yield of this process is quite good.

Dr. Rollefson: That would be a definite objection to my suggestion.

Dr. Kassel: It would probably be difficult for vibrational energy of HCO to help the reaction between the HCO groups to form hydrogen. Vibrational energy persists for perhaps a thousand collisions, but it is hard to imagine 0.001 of all the molecules at any time being HCO.

Dr. Kistiakowsky: The formation of glyoxal may be a serious objection to the mechanism discussed in my paper, although the HCO groups are supposed to be removed rapidly by their reaction with CH₃.

Dr. Noyes: Am I right in remembering Leermaker's results at room temperature, in which the photolysis of acetaldehyde seemed to take place partly by each of the two mechanisms?

Dr. Kistiakowsky: He had to assume, in order to explain the temperature coefficient, that not every molecule decomposes upon absorption of light.

Dr. Bates: There has been a recent paper by Terenin on the fluorescence of polyhalides which show the band fluorescence of halogen molecules. This he cites as proof of Norrish's theory of ketone decomposition. But the fluorescence could be due to recombination of halogen atoms.

Dr. Rollefson: The probability of getting fluorescence by a recombination process is very low. Kondratjeff has found that only one in 10⁸ collisions between halogen atoms results in recombination with the emission of light. Even under the most favorable conditions, the number of effective collisions probably would not exceed one in a million.

Dr. Noyes: For further argument, the fluorescence is proportional to the incident intensity and not to the square of it.

I believe West presented a paper, to the New York meeting of the Chemical Society, in which he found acetaldehyde to give free radicals by using the ortho-para hydrogen conversion. I think it is necessary, however, to assume that the CH₃CO group has a long life, unless the low quantum yield is ascribed to the primary process. One cannot get CO to combine with CH₃ groups to give acetone. I have tried it more or less foolishly, producing methyl groups photochemically from lead tetramethyl. The reaction was certainly much too slow to account for the low yield in acetone photolysis.

Dr. Kassel: Everyone who has used CH₃CO has wanted it to have a fairly long life for a variety of reasons.

Dr. Leermaker: In connection with Professor Kistiakowsky's interpretation of the mode of decomposition of long chain ketones, unpublished results on the high temperature photolysis of some aralkyl aldehydes and of valeraldehyde are very interesting.

I have studied the photodecomposition of phenylacetaldehyde, hydrocinnamic aldehyde and valeraldehyde at temperatures of 180° C. and with light of wavelength 3130Å. At this wavelength, the light is absorbed in each of these cases almost entirely by the carbonyl group, as Professor Kistiakowsky has shown; the primary photochemical process can safely be assumed to be the same as that occurring in acetaldehyde. The significant facts are that of the quantum yields of decomposition of these three compounds none is greater than unity at 180° C., whereas with acetaldehyde at this temperature they are of the order of 10 to 20.

The latter facts offer strong support to the idea that when radicals larger than methyl are liberated in the photolysis of ketones and aldehydes, these larger radicals are unstable and

suffer thermal degradation or, when possible, rearrange. Either the radicals are of insufficient activity to propagate a chain or they disappear; it seems improbable that at the temperature of the measurements there would be insufficient activation energy to allow the radicals to undergo thermal reactions, and we are led to believe that degradation occurs. With acetone it seems fairly certain that the methyl radicals do react with acetone at 180° , but that the product of such reaction is unstable and cannot enter into a chain.

It would be very interesting to study the thermal decomposition of azomethane in the presence of acetone. Azomethane is known to liberate methyl radicals on thermal decomposition, and these radicals should react with acetone to liberate methane, if my mechanism of the acetone photolysis is correct.

In connection with Professor Kistiakowsky's remarks on photochemical oxidation reactions, mention might be made of the excellent experiments of Bates and Spence on the oxidation of free methyl radicals. The radicals were liberated by photolysis of methyl iodide, and their subsequent history in the presence of oxygen was studied. It may be possible to obtain very interesting and illuminating results by photolyzing acetaldehyde in the presence of oxygen, and carefully studying the reaction products and the mechanism of the reaction. Some correspondence between the course of such reaction and the course of the reaction studied by Bates and Spence might be expected.

Dr. Bäckström: I quite agree with Kistiakowsky that the change of an aldehyde molecule upon light absorption into a radical with a free valence on the oxygen and another on the carbon represents nothing but an electronic shift and should manifest itself by the appearance of discrete bands in the absorption spectrum. However, just this type of spectrum is shown by the aldehydes in the near ultraviolet, and I am sure that Kistiakowsky is aware of this fact, although he does not specifically mention it except in the case of formaldehyde. Therefore in this wavelength region, which is highly active in promoting the oxidation of the aldehyde, there can be no question of a primary dissociation of the light absorbing molecule; but, if I have understood Kistiakowsky correctly, he prefers to assume that a dissociation takes place as a secondary process on collision with other molecules.

This possibility, however, seems to me to be effectively excluded by the available experimental evidence. In the case of benzaldehyde, for instance, we know from the work of de Hemptinne that wavelengths below 2700 \AA cause benzaldehyde vapour to decompose into C_6H_6 and CO , whereas no such effect is obtained on irradiation

with longer wavelengths. From my own experience I may state that liquid benzaldehyde, which is sealed up in a glass tube, may be exposed to the sun or a mercury arc until more than half of the aldehyde has been transformed into polymerization products, without any gas pressure being noticed on opening the tube. There is thus no sign of dissociation under these conditions. From experiments in the presence of oxygen, on the other hand, we know that the wavelengths which pass through glass are at least as effective in starting oxidation chains as the shorter wavelengths.

These are some of the facts upon which my conception of the reaction mechanism is based; others are given in a paper in *Z. phys. Chem.* (ref. 28).

As emphasized by Kistiakowsky, the region of sharp bands, which in the case of the aliphatic aldehydes is rather short, is succeeded on the short wavelength side by a region of predissociation bands. If oxygen is present, illumination with light within this region gives rise to oxidation chains. From the character of the spectrum, one is justified, in this case, in assuming that the primary act is a dissociation of some kind, and I wish to point out, that a dissociation, $\text{RCHO} = \text{RC}'\text{O} + \text{H}$, as discussed by Kistiakowsky, would fit in very well with the chain mechanism which I have proposed, since this mechanism is based on the reactions of acyl radicals. However, there seems to be a distinct possibility that in this case, as well, the primary act is the formation of a radical $\text{RC}'\text{HO}$ — with two free valences; the only difference being that with decreasing wavelength, i.e. with increasing vibrational energy, this radical becomes increasingly unstable and more short-lived.

Kistiakowsky points out, in discussing the predissociation state of the molecule, that "since the time between absorption of light and decomposition is here appreciable and may be even longer than the average time between molecular collisions at moderate gas pressures, it is conceivable that the activated molecule may undergo some other reaction on collision instead of decomposition, or even lose its energy in this manner. These considerations apply, of course, particularly to reactions taking place in condensed phases."

In discussing the photodecomposition of the aldehydes, however, he seems to forget about the former of these possibilities, since he mentions only the latter. This seems the more surprising as in this case a side-reaction is actually known to take place, namely, a polymerization of the aldehyde. In the work of Leighton and Blacet on the photodecomposition of acetaldehyde, they also determined the quantum yield of polymerization and found that this yield increases with increasing wavelength. At a pressure of about 200 mm.

they obtained values of 0.1 and 0.47 at 2537 Å and 3130 Å, respectively. Under the same conditions the quantum yield of decomposition decreased from 0.9 to 0.2. Experiments at the latter wavelength showed that with increasing aldehyde pressure the rate of polymerization increases whereas the rate of decomposition decreases. It is obvious, therefore, that there is a competition between these two reactions, which accounts for at least part of the deficit in the quantum yield of decomposition. Judging by the figures of Leighton and Blacet, the decomposition yield in liquid aldehyde may be expected to be very small indeed; and I am inclined to the belief, therefore, that under these conditions, at least, the chains in the oxidation reaction are started in the same

manner in the predissociation region as in the region of sharp bands, i.e. by a reaction between a normal and an excited aldehyde molecule.

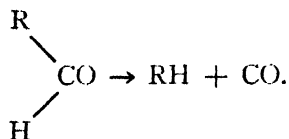
As regards the Haber-Willstätter chain mechanism of aldehyde oxidation, to which Kistiakowsky refers in his paper, it seems to me that this reaction scheme might as well be left out of the discussion until somebody has shown how it can be modified to give the reaction products actually formed; in the case of benzaldehyde, for instance, benzoperacid and not benzoic acid. This fact, that the two oxygen atoms of the oxygen molecule are still linked to each other in the reaction product, seems to me to form the strongest possible argument against the appearance of hydroxyl radicals in the reaction chain.

COMBINED DISCUSSION OF PAPERS BY PROFESSORS KISTIAKOWSKY AND ROLLEFSON WITH SPECIAL REFERENCE TO THEIR BEARING ON THE PHOTOCHEMICAL DECOMPOSITION OF CARBONYL COMPOUNDS

R. G. W. NORRISH

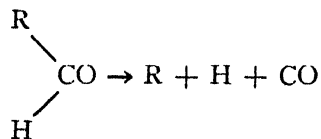
The questions raised by Professors Kistiakowsky and Rollefson in their introductory papers are very interesting to me. In discussing their papers, however, I must limit myself to referring to those points on which we find ourselves in disagreement, pausing only to express my admiration of their masterly presentation of the main thesis. Their papers undoubtedly put in a very clear way the principles which are guiding photochemists in their present problems.

When we first drew attention in 1931⁽¹⁾ to what appeared an interesting point in the decomposition of aldehydes, we were limited entirely by the published data, which at that time suggested to us that the *primary* process is represented by the reaction:

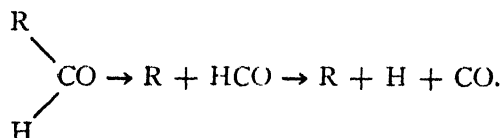


Our conclusion was confined to aldehydes and we made no pronouncement about ketones for which no suitable data were in existence. Since that time, the work of Leighton and Blacet⁽²⁾ on acetaldehyde and propionaldehyde has confirmed our view that between 90% and 100% of the aldehyde decomposes to give a single hydrocarbon. (Exception must be made of one isolated observation which widens these limits to 80-100%). For reasons given below we cannot agree with Kistiakowsky when he says that "A primary formation of carbon monoxide and a stable hydrocarbon seems to be out of the ques-

tion because of the observations of Leermakers⁽³⁾ at higher temperatures." It should be noted that Leermakers himself says in his paper⁽⁴⁾ that "it is possible there are two modes of decomposition of aldehydes, one resulting in radicals, and the other carbon monoxide and hydrocarbon directly". To this view, as a result of our studies with ketones we have ourselves for some time been strongly inclined, and we believe that by accepting it a great simplicity of hypothesis is achieved. To account for the chain reactions at high temperatures, and for the small quantities of hydrogen observed at low temperatures, it is only necessary to suppose that a small proportion (less than 10%) of the aldehyde decomposes in the way found by us for short chain ketones, i.e.:



or



In agreement with this view, and in disagreement with the view expressed by Kistiakowsky it should be mentioned that Locker and Patat⁽⁵⁾ have been forced to the conclusion that *molecules* and not free radicals are produced from formaldehyde both in the region of fine structure and

in the region of diffuse bands below $280\text{ m}\mu$, while their results at still shorter wavelengths in the continuum are inconclusive owing to the direct oxidation of the formaldehyde to formic acid and the photodecomposition of the latter to water and carbon monoxide.

Not only do the experiments of Locker and Patat support our view but also those of Pearson⁽⁶⁾ and we do not subscribe to the view that free hydrogen atoms and free hydrocarbon radicals would necessarily combine more rapidly than free hydrocarbon radicals themselves—certainly Rice⁽⁷⁾ appears to have experienced no difficulty in the demonstration of the thermal production of free radicals from aldehydes by the Paneth method, and if they are similarly formed photochemically to any appreciable extent they would have, in our view, been detected.

Moreover the primary mechanism involving the formation of the $\cdot\text{CHO}$ radical from formaldehyde, and other aldehydes is difficult to reconcile with the statement made earlier in Kistiakowsky's paper that this radical yields formaldehyde and glyoxal in varying proportions. The fact that the carbon monoxide is always produced in slight excess over the hydrogen in the decomposition of formaldehyde leaves little room for the polymerisation of this radical to glyoxal, and we conclude that it is not present in appreciable quantity.

But apart from this, the quantitative data for formaldehyde are strongly against the production of free hydrogen atoms. The quantum yield remains unity over the range of wavelengths $250\text{--}330\text{ m}\mu$ ⁽⁸⁾. There is no fluorescence between these limits, so we may conclude that throughout we are in a region of predissociation. Now the C-H bond is generally held to be of the order 100 k.cal , while the magnitude of the quantum at $330\text{ m}\mu$ is not greater than about 86 k.cal , and it is not until we reach a wavelength of about $280\text{ m}\mu$ that the primary act postulated by Kistiakowsky can occur.

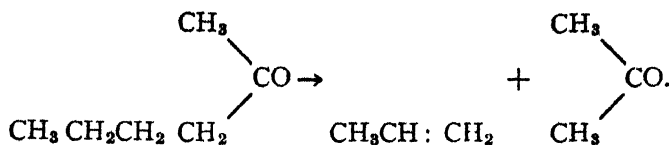
Turning now to our studies with ketones, we believe that the results with methyl ethyl ketone and with acetone, as accepted by Leermakers⁽⁹⁾, prove beyond doubt the formation of free methyl and ethyl. The conclusions from the results of our detailed analysis have been confirmed by Pearson⁽⁷⁾ and we cannot agree with Professor Rollefson that there is any discrepancy with the analytical data of other workers serious enough to throw doubt on these conclusions. Such discrepancies as exist are unimportant to the main issue, but we would emphasise the fact that in all our analyses we have carefully fractionated both gaseous and liquid products, and in nearly all cases succeeded in carrying out separate estimations of each product. The data of nearly all

earlier workers are based on indirect analysis by explosion, and as such they cannot lay claim to the same accuracy.

Our results with ketones do not show whether the two free radicals are produced in one act, or the one rapidly after the other due to the instability of the acyl radical, but we consider that the absence of any $\alpha\alpha$ -dicarbonyl product is evidence against the existence of any comparatively stable acyl radical. The fact that aldehydes however give in the main a single hydrocarbon now inclines us to the view that the severance is simultaneous and not successive. The idea brings with it an economy of hypothesis which to our mind is refreshing after trying to adjust the velocities of the manifold hypothetical reactions of atoms and radicals which have been suggested, —to give the right answer. All that is necessary is to postulate a change in the multiplicity of the carbonyl group, from a triplet to singlet state. In this way carbon monoxide is a primary product and the two free radicals are left "high and dry". The energy required for the whole change is not greater than 80 k.cal . and agrees in general with the threshold of photochemical activity in the spectrum. It is then not difficult to imagine that in the case of aldehydes the greater mobility and proximity of the hydrogen atom to the alkyl radical nearly always results in the primary formation of a hydrocarbon, while with ketones, considerations of steric hindrance, and the relative immobility of the alkyl radicals result in their escaping separately from the decomposing molecule.

Our results with cyclic ketones⁽¹¹⁾ are in full accord with this view and we appreciate the fact that Professor Kistiakowsky has laid stress on the difficulty that these substances create for his theory. If other acyl radicals are stable enough to wait for chance recombinations in the gas phase, it is difficult to see why free acyl radical produced by the rupture of a cyclic ketone should not be similarly stable. Yet in spite of the fact that the chance of recombination in this case must be much greater, since it is located in the ruptured molecule itself, Kistiakowsky is driven to assuming that there is a rapid and spontaneous decomposition by the liberation of carbon monoxide, before such recombination can occur. In short, in the case of cyclic ketones, he comes precisely to our declared way of thinking in an endeavor to avoid the consequences of his own theory.

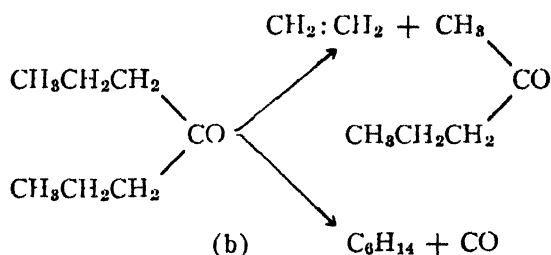
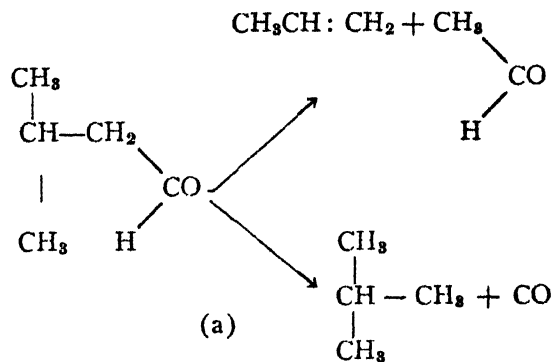
With aldehydes and ketones with long chains⁽¹⁰⁾ we have found what we conclude to be an entirely different primary mechanism of decomposition. Thus, with methyl butyl ketone more than 90% of the reaction follows the course:



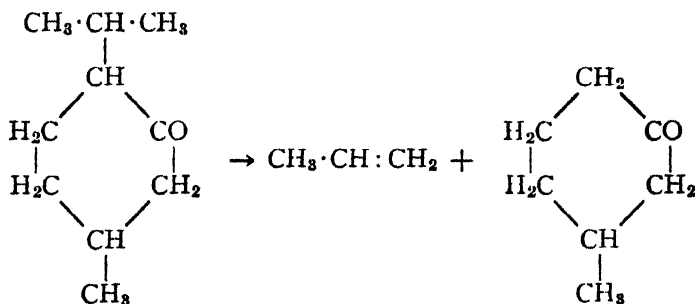
This we regard as the primary change, and conclude that it is preceded by a transfer of energy from the chromophoric group (the carbonyl group) to the point of reaction. Professor Kistiakowsky on the contrary seeks to explain the results on the assumption that free butyl radicals are first liberated; that these decompose to propylene and methyl, and that the methyl and acyl groups recombine to yield the acetone. It is extremely doubtful however if this mechanism could give rise to a yield of acetone, nearly equivalent to the propylene such as we find, and we should certainly expect to find greater quantities of ethane, and carbon monoxide than actually appear. If I may be permitted to refer to unpublished work I would cite the experiments of my collaborator Bloch who has recently studied the same decomposition in approximately monochromatic light at a temperature of 127°C. He finds the proportions of the products unchanged; thus at a temperature approaching that suggested by Kistiakowsky as a crucial test, the evidence is against his theory.

His theory cannot be sustained however on other grounds. With the vapour of menthone Bamford and I have recently found that the main primary decomposition may be represented as

be about equally distributed between the two types i.e:



All the products have been isolated in a quantitative manner and the full analytical results upon



It would seem to strain the free radical theory too much to account for this change, for no rupture of either carbonyl bond could conceivably give rise to these products. On the other hand the hypothesis of energy transfer from one part of the molecule to the other provides a simple and adequate explanation of the analytical data.

In our view this type of change will be found to be at least as frequent as that by which the simpler carbonyl compounds decompose. We have found that it holds both for iso-valeric aldehyde (a) and for dipropyl ketone (b) though here the probability of decomposition appears to

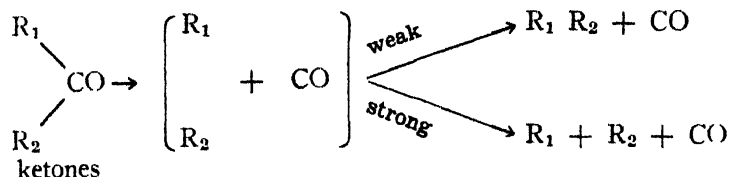
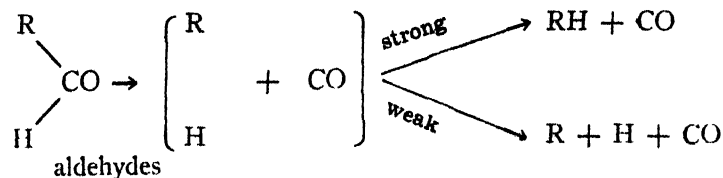
which we base these equations will be published at an early date.

It will be seen that in the four cases studied, i.e. methyl butyl ketone, iso-valeric aldehyde, dipropyl ketone, and menthone, the primary rupture occurs between carbon atoms lying in positions α and β to the carbonyl group. So far then this appears to be a general rule for this type of decomposition, and we thus have the additional simplicity that in four molecules of quite varied shape our energy transfer has taken place in precisely the same way. If it is sought to explain

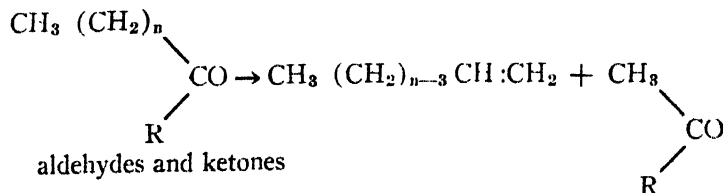
these reactions in term of free radicals, then it will be necessary to account for this uniformity of behaviour.

To recapitulate: the decomposition of carbonyl compounds may be explained on the basis of two types of primary reaction:

Type (1);



Type (2);



Type (1) predominates in the case of carbonyl compounds with short chains, type (2) in the case of carbonyl compounds with long chains, but type (1) persists longer in the aldehyde than in the ketone series.

Given these two primary reactions all the extensive analytical data are satisfactorily explained, while the direct experiments of Locker and Patat⁽⁵⁾ and Pearson⁽⁶⁾ with formaldehyde, acetone, methyl ethyl ketone and methyl butyl ketone are in full agreement.

For the reasons stated above it is not apparent that the same advantages can be claimed by those who would press for an explanation of all the facts entirely in terms of free radicals; and while fully admitting the importance of free radicals in the study of chemical reactivity we consider it unjustifiable in the present instance to take the extreme view that their reactions (in many cases hypothetical) can be responsible for all the observed facts. The question is controversial: if the extreme exponents of free radicalism can deal satisfactorily with the objections raised above it will remain so. If they cannot they are in a weak position.

For ourselves, we prefer the *via media* and, while admitting the increasing importance of the reactions of radicals at the higher temperatures, see no reason to abandon our theory until some fact arises to disprove it.

I regret that lack of space and time prevent

any further reference on my part to many of the other views expressed by Professor Rollefson in his interesting paper. Some of the points raised by him however, I have further discussed in a forthcoming paper in the "Acta Physico-chimica" of the Soviet Union.

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THE CHEMICAL PROPERTIES OF X-RAY ACTIVATED MOLECULES WITH SPECIAL REFERENCE TO THE WATER MOLECULE

HUGO FRICKE

Studies of the influence of X-rays on vital processes have, in many cases, been brought to the point where speculations based on general chemical principles can be made profitably. The need for such a general point of view is especially felt by the biologist when he compares the biological effects of X-rays with those of light, and attempts to correlate the similarities and differences between these two agencies. The genetic effects of radiation form a favorable field for such theoretical considerations, for we observe here changes assumed to be referable to changes in a single structure of molecular dimensions, the gene, or perhaps even to a change in a comparatively small molecular group of such a structure.

According to the present ideas of photochemistry, the difference in chemical effects produced by X-rays and by light of a particular wavelength lies in the difference between the types of activated states produced in the two cases, those produced by X-rays being generally at higher energy levels than those produced by light. A considerable number of the activated states produced by X-rays involve ionisation, as may be concluded from the high conductance acquired by gases irradiated with X-rays. The number of ion pairs produced by the absorption of a fixed amount of X-ray energy is nearly the same for all gases, and is at the rate of one pair for each thirty electron-volts of energy absorbed. The minimum energy required for the ionisation of a molecule is of the order of fifteen electron-volts. The difference represents energy expended in the production of states below the point of ionisation, and the excess energy required to produce states beyond the point of ionisation. We may conclude that at least half of the absorbed X-ray energy is expended in the production of ions, while at the most half of the energy is used in producing non-ionised states. This conclusion as to the prevalence of ionised states is in keeping with the fact that when endothermic reactions are produced by irradiation with X-rays, the number of molecules transformed is generally of the same order of magnitude as the number of ion pairs produced by absorption of the same amount of X-ray energy in a gas.

Our knowledge of the lower states of activation of molecules is chiefly derived from spectrographic evidence, as has been described earlier in this symposium. Limiting ourselves to a very schematic presentation, we may say that molecules can take up energy by two methods. In the first place we have the energy of the movement of the

molecule as a whole, translational and rotational, and the energy of the vibrations of the various parts, atoms and atomic groups, in respect to each other. In the second place, with each valence electron there is associated a certain number of activated states, corresponding to the removal of the electron to its various quantum orbits. These states are reflected in the absorption spectrum of the molecule as a series of bands. The limiting state in this series is that in which the electron is completely removed from the molecule, this being the lowest state of ionisation. The energy required for ionisation, however, always exceeds that required for the dissociation of the molecule into its uncharged components. Before ionisation occurs, states are reached in which the activation of the molecule leads to its dissociation, this being shown in the spectrum by a region of continuous absorption. As we supply the molecule with amounts of energy in excess of that required for ionisation, either the surplus energy may be utilized as kinetic energy of the liberated electron, or excitation of the ionised molecule may occur. Since the corresponding wavelengths are situated in a very inaccessible part of the spec-

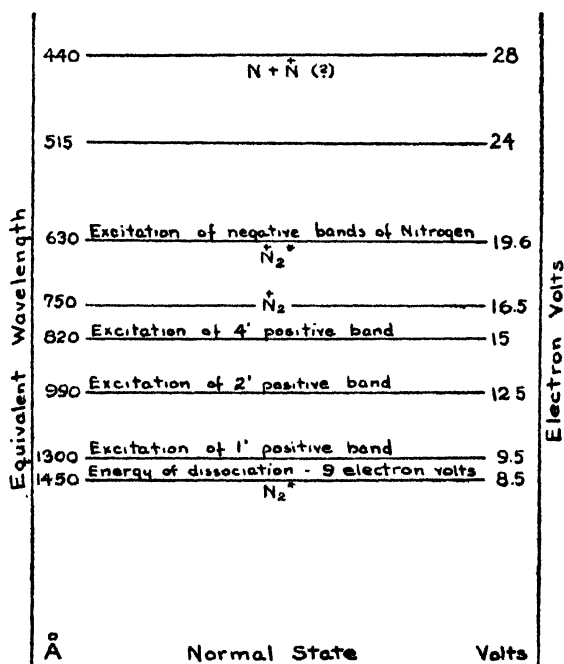


Fig. 1. Activated states of the nitrogen molecule, as determined by measurements of critical potentials. The energy of the various states is given in electron volts and in equivalent wavelengths.

TABLE
A Comparison of Chemical Effects Produced

IRRADIATED SYSTEM	α -RAYS		WAVELENGTH (A)
	REACTION	M/N*	
H ₂ O	None		
O ₂	3O ₂ = 2O ₃	3(O ₃)	2530 (av.) 1750 - 2070
CO ₂	None		
CO	CO ₂ + C + solid suboxides of carbon (C ₈ O ₂) _x	1-3 (CO)	
NH ₃	2NH ₃ = N ₂ + 3H ₂	1-3 (NH ₃)	2100
CH ₄	2CH ₄ = C ₂ H ₆ + H ₂ xCH ₄ = (CH ₂) _x (liquid) + xH ₂	about 2 (CH ₄)	
CH ₂ :CH ₂	Chief reaction: polymerization, (CH ₂) _x being formed (liquid); hydrogen and methane also formed in small amounts.	5 (C ₂ H ₄)	2000
CH:CH	Polymerization. (CH) _x (solid) formed. (Cuprene). Also small amount of H ₂ .	20 (C ₂ H ₂)	2050 - 2200
C _n H _{2n+2}	Chiefly hydrogen; smaller amounts of methane and other lower and higher hydrocarbons.	about 2 (C _n H _{2n+2})	
H ₂ + O ₂	2H ₂ + O ₂ = 2H ₂ O (Small amounts of H ₂ O ₂ also found.)	4 (H ₂ O)	Sensitization with Hg 2537 1860 1720
CO ₂ + H ₂	CO ₂ + 2H ₂ = H ₂ O + (H ₂ CO) _x (white, wax-like solid)	1.7 (CO ₂ + H ₂)	
CO ₂ + CH ₄	(H ₂ CO) _x (white, wax-like solid)	.75 (CO ₂ + CH ₄)	
CO + H ₂	(H ₂ CO) _x (white, wax-like solid)	3.3 (CO + H ₂)	Sensitization with Hg 2537
N ₂ + H ₂	N ₂ + 3H ₂ = 2NH ₃	.2 (NH ₃)	

* M/N = ratio of mols transformed, or produced, to number of ion pairs.

trum, usually below 1000 A, we have little knowledge of these states from spectroscopic evidence, but our information on this subject is chiefly derived from electrical measurements in which the result of collisions between electrons and gas

molecules is studied as a function of the electronic energy.⁽¹⁾

In Fig. 1 are shown activated states of the nitrogen molecule, as determined by this method. The ionisation potential of nitrogen is 16.5 volts,

I.

by Irradiating Gases with α -Rays and Light.

LIGHT			REMARKS
TYPE OF ACTIVATION	REACTION	QUANTUM EFFICIENCY	
	No data		
O_2^* $O + O^*$	$3O_2 = 2O_3$	1.5 (O_3) 3 (O_3)	
	No data		
	No data		
$NH_2 + H(?)$	$2NH_3 = N_2 + 3H_2$.25 (NH_3)	
	No data		
	$CH_2:CH_2 = CH:CH + H_2$		
	Polymerization. (CH) _x formed. (Cuprene?)	9 (C_2H_2)	
	No data		
$H + H$ O_2^* $O + O$	$H_2 + O_2 = H_2O_2$ O_3 dominates—also H_2O_2 ($8H_2O?$) O_3 , H_2O_2 and H_2O are formed.	1.2 (H_2O_2) Quantum yield depends on ratio of pressures of O_2 and H_2 , in a manner theoretically explainable.	
	No data		
	No data		
$H_2 = H + H$	$CO + H_2 = H_2CO$ $2CO + H_2 = (HCO)_2$	1.4 (aldehyde groups)	
	No data		Ammonia is not ⁽⁴⁾ produced by passing electrons of up to 30 electronic volts through $N_2 + H_2$ (at low temperature).

at which potential N_2^+ is produced. At potentials around 19 volts excited states of N_2^+ are obtained, giving rise to the emission of the so-called negative bands of nitrogen. At 28 volts, there is some evidence that N^+ is produced.

The states below and including dissociation are usually within the accessible parts of the spectrum, and our knowledge of the chemical properties of molecules in these states is comparatively extensive. While some reactions are known

which are associated with molecules in activated states below that of dissociation, as for example, the production of ozone from oxygen by irradiation with 2500 Å⁽²⁾, most known photochemical reactions take place as the result of dissociation. At this point, free atoms and radicals are produced, and changes occur which are the result of their rearrangement in new molecules. An important point as to specific chemical effects produced with X-rays is to what extent the transfer of amounts of energy to a molecule beyond that required for dissociation leads to the occurrence of new types of chemical reactions; but at present this cannot be answered. The experimental difficulties in imparting such energies by means of light are great, since the required wavelengths usually are below 1000 Å.

The chemical properties of molecules in their higher states of activation have been studied by excitation with alpha-rays from radium⁽³⁾, in electric discharges such as the spark, arc and Geissler tubes, in addition to X-rays. There are also a few investigations of the chemical reactions produced by bombarding gases with electrons of known velocities, in particular on formation of ammonia from nitrogen and hydrogen^{(4) (5)}. One may hope for important results from this method, but so far the interpretation of the results obtained is not clear.

Lind and his collaborators⁽³⁾ have carried out a very extensive work on the chemical reactions produced with alpha-rays, and in Table 1 are given the results of some of his experiments and a comparison with results obtained by the use of light. The table shows the general similarity of the transformations produced by these two agencies. In such cases where the effects produced by light change qualitatively with wavelength, there is a greater similarity, as is to be expected, between the effects of alpha-rays and of light of the shorter wavelengths. It may be noted that neither water nor carbon dioxide can be decomposed with alpha-rays. A difference is shown between the effects of alpha-rays and light, in the case of the irradiation of ethylene. With alpha-rays, a liquid polymerization product is formed with only small amounts of hydrogen appearing, while with light around 2000 Å, the transformation of the ethylene to acetylene and hydrogen follows.

In the case of X-rays, systematic work on their chemical effects has been carried out for aqueous solutions only. Gas-free water is not changed by irradiation with X-rays⁽⁶⁾; the X-rays, however, cause an activation of the water molecules, as is shown by the fact that irradiation can bring about the chemical transformation of substances present in the water in such a high dilution that their direct activation by the rays is negligible.

Particularly simple results have been obtained for certain compounds capable of adding an oxygen atom, including the nitrite, arsenite and selenite ions. Irradiation of a solution of any one of these molecules causes its oxidation, and hydrogen is produced in the equivalent amount. For example, for the nitrite ion, the reaction may be written

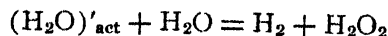


For a dosage of 1000 r, and per 1000 cc. of solution* for each of these molecules 0.55 micromols are transformed, irrespective of the concentration of the solution, and of the hydrogen ion concentration.

In attempting to bring the various reactions which have been studied into a connected scheme, one may postulate the production of different types of activated water molecules. We can assume that the reactions just discussed are caused by one particular type of activated molecule designated by $(\text{H}_2\text{O})'_{\text{act}}$, this molecule being produced at the rate of 0.55 micromols per 1000 cc. and per 1000 r.

The finding that X-rays do not decompose water came rather as a surprise, since there are experiments on record in which water has been decomposed by irradiation with α-rays⁽⁷⁾. A possible explanation of this apparent inconsistency is suggested by recent experiments in which it is found that water can be decomposed by X-rays in the presence of certain molecules acting catalytically. We have particularly investigated this decomposition in the case of the iodide ion.⁽¹⁷⁾ The reaction occurring depends on the hydrogen ion concentration. In acid solution, hydrogen and hydrogen peroxide are produced; in basic solution, the same amount of hydrogen is obtained, but instead of hydrogen peroxide, oxygen is produced in an amount corresponding to the decomposition of the peroxide into oxygen and water. The amount of hydrogen produced is 0.55 micromols per 1000 cc. and per 1000 r. There is no observable change in the concentration of the iodide.

The heat of reaction for the decomposition of two gram molecules of water into one gram molecule of hydrogen and one gram molecule of hydrogen peroxide, is 91 Cal. Since the rate of decomposition is .55 micromols of $\text{H}_2/1000 \text{ cc} \times 1000 \text{ r}$, we may assume that the decomposition takes place according to:



* A dosage of 1 r unit produces an ionisation of 1 electrostatic unit in 1 cc. of atmospheric air at 0°C. and 760 mm.

and conclude that the energy content of $(\text{H}_2\text{O})'_{\text{act}}$ is at least 91 Cal. per gr. molecule.

Besides $(\text{H}_2\text{O})'_{\text{act}}$, our work indicates the production of at least one other species of activated water molecule, designated $(\text{H}_2\text{O})''_{\text{act}}$, and formed in the amount of 2.2 micromols per 1000 cc. \times 1000 r. This molecule has the particular ability of bringing the oxygen molecule into a reactive state. As an example, we may mention the action of X-rays in solutions of ferrous sulphate^{(9) (18)}. In gas free solution, the ferrous ion is oxidized to the ferric ion, with the equivalent production of hydrogen, and at a rate depending on the hydrogen ion concentration, as shown in Figure 2. If oxygen is present, the quantity of

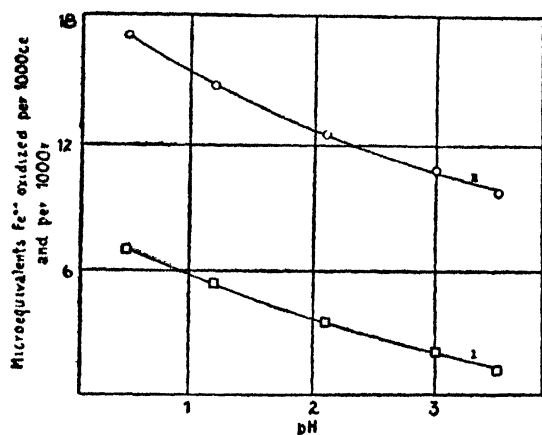


Figure 2.* The oxidation of ferrous sulfate in sulfuric acid as a function of the pH of the irradiated solution; I. gas free; II. oxygen present.

ferrous ion oxidized is increased by a fixed amount: 8.8 micro-equivalents per 1000 cc. and per 1000 r. For this increased oxidation $(\text{H}_2\text{O})''_{\text{act}}$ is assumed to be responsible, each $(\text{H}_2\text{O})''_{\text{act}}$ activating one oxygen molecule, leading, for each $(\text{H}_2\text{O})''_{\text{act}}$, to the oxidation of 4 equivalents of ferrous sulphate.

It is of interest to compare the numbers of activated water molecules with the number of ion pairs which would be produced in the water if we could collect all the ions. This we cannot do in practice, but we may refer to the ionization produced in the same quantity of water vapor. This is not known by actual experiment either, but since it is found that the ionizations produced by absorbing a fixed quantity of X-rays by a number of gases, including hydrogen, oxygen and nitrogen, are nearly the same, we may assume that the ionization would be the same also for

water vapor. On this assumption we calculate that a dosage of 1000 r produces 2.8×10^{-6} gram ion pairs in 1000 g. of water.

I shall now select for discussion a few of the reactions which we have studied and which are particularly interesting from the point of view of the biological effects of X-rays. Since 60 or 70% of the cell is water, we should expect that the reactions due to the activation of the water would play an important role in the changes produced by the rays, in addition to the changes due to the direct X-ray activation of the other constituents of the cell.

It has often been suggested that a primary production of hydrogen peroxide by the X-rays is in part responsible for their biological effects. Irradiation of water containing gaseous oxygen causes the production of hydrogen peroxide,⁽¹⁹⁾ the amount produced being independent of the oxygen pressure, down to 4 cm. of Hg., and dependent on the hydrogen ion concentration, as shown in figure 3, where dosage curves are given

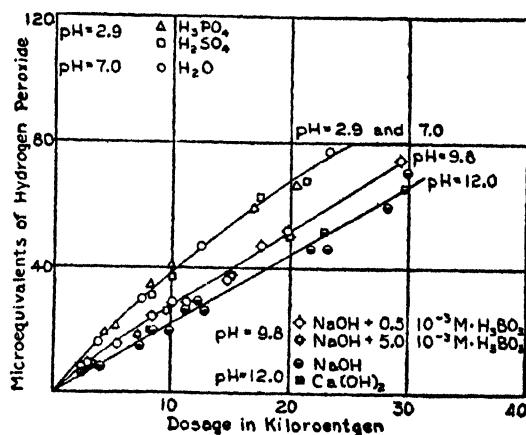


Figure 3. The production of hydrogen peroxide as a function of X-ray dosage for different values of the hydrogen ion concentration.

which represent the results of irradiating solutions of pH ranging from 2.0 to 12.0. The deviation of the dosage curves from linearity, in the low pH range, is due to the decomposition of the hydrogen peroxide by the rays. At higher dosages, the curves all become straight, with the same slope as observed in basic solutions and the amount of hydrogen peroxide produced in this range is 1.1 micromols/1000 cc. \times 1000 r. If each oxygen molecule by activation produces 2 molecules of hydrogen peroxide, the number of oxygen molecules activated is 0.55 micromols ($(\text{H}_2\text{O})'_{\text{act}}$). It may be observed that an influence of the hydrogen ion concentration is common to a number of reactions and in general we find that X-rays exert greater chemical activity in acid than in basic solution.

* Figures 2, 3, 5, 6, and 7 have appeared in the Journal of Chemical Physics, and are used here with the permission of the editor of that journal.

Certain of the reactions occurring when solutions containing free oxygen are irradiated, may be considered as due to a primary production of hydrogen peroxide. However, there are a number of reactions produced by X-rays which cannot be produced by adding hydrogen peroxide. In some cases we find that the irradiation induces reactions which can also be obtained with hydrogen peroxide if we give the hydrogen peroxide sufficient time to act. This is shown by the results⁽²⁰⁾ in figure 4. If we irradiate potassium

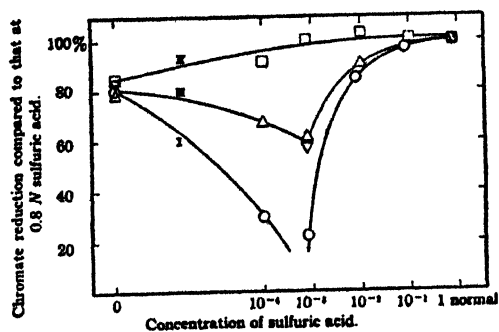


Figure 4. The reduction of chromate by X-rays as a function of acidity. Curve I, \circ , irradiation of N/9000 chromate; Curve II, \square , irradiation of sulfuric acid and addition of chromate and strong acid immediately; Curve III, \triangle , irradiation of sulfuric acid, addition of chromate immediately, and of strong acid sixteen hours later; Curve IV, ∇ , addition of hydrogen peroxide to N/9000 chromate and of strong acid sixteen hours later.

bichromate in strong acid solution (pH smaller than 1) and in the presence of free oxygen, the chromate is reduced to the same extent as obtained when we irradiate the solution without the bichromate (thereby producing hydrogen peroxide), and thereafter add this irradiated solution to the bichromate. At a pH between 3 and 4, irradiation of a solution of bichromate has no effect. If at this pH, we add hydrogen peroxide to the solution of bichromate we find that there is no immediate action. However, very slowly, over a period of days, the hydrogen peroxide decomposes with no change of the bichromate. At this particular pH, the bichromate causes the catalytic decomposition of the hydrogen peroxide; evidently, therefore, we may explain the lack of action of the X-rays on bichromate at a pH of 3.5 by assuming that hydrogen peroxide is produced initially in a highly reactive form which allows its immediate decomposition by the bichromate.

The decomposition of hydrogen peroxide by X-rays introduces another principle which has an important bearing on the action of the rays on

the cell. In this reaction, a dependence on the X-ray intensity is found to exist.⁽²¹⁾ In all other reactions which we have investigated, the transformations produced are dependent solely on the total dosage given, independent of the intensity of, or of the time used for, the irradiation. As shown in figure 5, the amount of hydrogen perox-

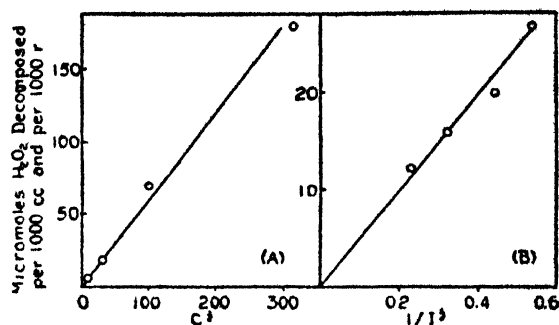
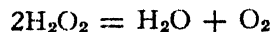


Figure 5. Decomposition of hydrogen peroxide as a function of: (A) Square root of concentration (C in micromols per 1000 cc.) for an intensity of 9.5 r/sec. (B) Inverse square root of intensity (I in r/sec.) for a concentration of 1.0 millimol H_2O_2 per 1000 cc.

ide decomposed per unit of dosage varies inversely as the square root of the X-ray intensity. It will also be noted that the rate of decomposition increases as the square root of the hydrogen peroxide concentration. The decomposition proceeds according to



with no production of hydrogen.

A dependence of the chemical effect of X-rays on their intensity will probably only be found for exothermic reactions. For these, such a dependence may not be unusual, although for the few exothermic reactions which we have studied besides the decomposition of hydrogen peroxide, we have found no dependence on the intensity. It is a fact, very important for the therapeutic usage of X-rays, that their biological effects in certain cases show dependence on the intensity. It has usually been assumed that this dependence was due to a change of the biological material during the irradiation, but the results obtained for the decomposition of hydrogen peroxide show that a dependence of the primary chemical reactions on the intensity may also be responsible.

The decomposition of hydrogen peroxide is also the only reaction we have studied which shows an appreciable dependence on the temperature.⁽²¹⁾ (Fig. 6).

The description of results obtained in irradiating solutions of organic materials is particularly pertinent. A considerable number of organic

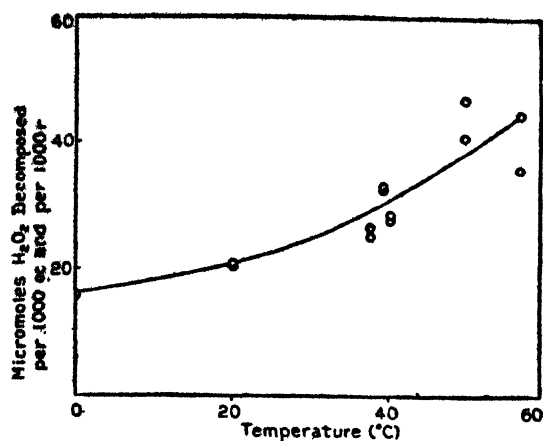


Figure 6. Decomposition of hydrogen peroxide as a function of the temperature for an X-ray intensity of 3.0 r/sec. and a concentration of 1.0 millimol H_2O_2 per 1000 cc.

molecules, particularly those of the aliphatic series, have been studied^{(17) (22) (24)}. In all cases, hydrogen is produced, and for certain highly oxidized types of molecules, carbon dioxide is also obtained. The amount of these gases produced usually increases with the concentration, indicating the occurrence of secondary reactions. However, the primary reaction would appear to consist in the attachment of the oxygen of the activated water molecule to the organic molecule.

Particularly simple results were obtained for formic acid.⁽²²⁾ The reaction depends on the hydrogen ion concentration. In acid solution (Fig. 7), equal amounts of hydrogen and carbon diox-

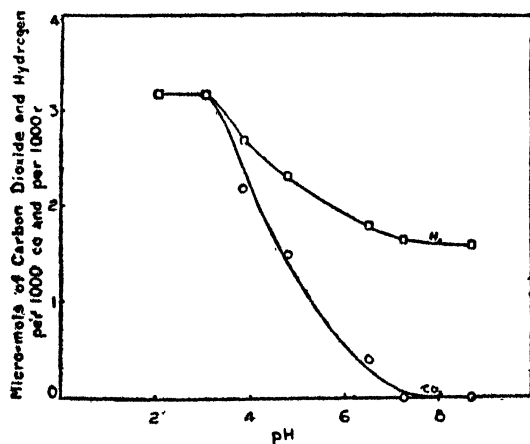
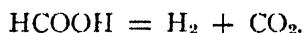
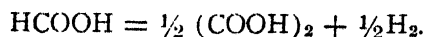


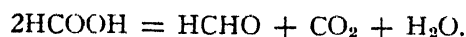
Figure 7. The production of carbon dioxide and hydrogen as functions of the pH of the irradiated solution.

ide are produced, corresponding to the simple decomposition.

For larger values of the pH, the hydrogen production is reduced to one-half, while no carbon dioxide is produced. Probably oxalic acid is formed according to



In addition to these transformations, at high concentration of formic acid an additional reaction sets in, shown by an increased production of carbon dioxide. The production of hydrogen remains constant at all concentrations. This additional reaction appears to be a chain reaction and may perhaps be the reduction of formic acid to formaldehyde according to



Also for oxalic acid,⁽¹⁷⁾ within certain ranges of concentration and hydrogen ion concentration,

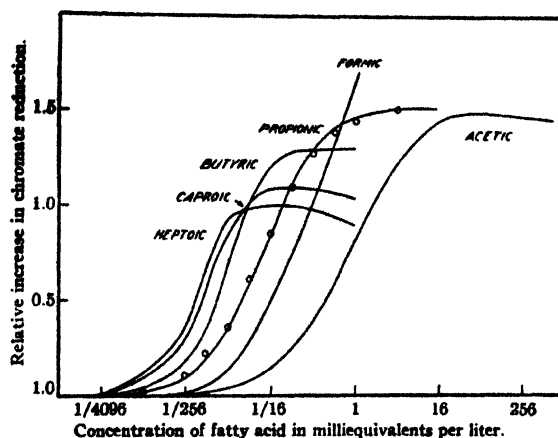
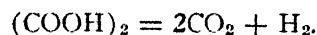


Figure 8. The reduction of chromate by X-rays as increased by the presence of fatty acids. The circles are calculated for propionic acid from $1.52C/(0.000052 + C)$.

irradiation results in a simple decomposition, according to



In the presence of oxygen, no hydrogen results from the irradiation of organic substances with X-rays, but there is an increase in the hydrogen peroxide produced.⁽²⁰⁾ In Fig. 8 are results obtained for certain of the monobasic acids of the paraffin series. The experiments were carried out in the presence of 0.8N sulfuric acid. The production of hydrogen peroxide was determined by the reduction of bichromate present in the irradiated solution.

It would be of considerable importance to determine the length of life of the activated water

molecules, the existence of which we have postulated in the foregoing. The method one would use for this purpose would be to study the reactions of the X-rays at very low concentrations of the reacting substances. When the concentration is reduced to a point where the activated water molecules responsible for the reaction do not encounter molecules of the reacting substance before returning to the normal state, then the number of molecules transformed would show a decrease. We have attempted to carry out such a study for the catalytic decomposition of water in the presence of potassium iodide. As noted above, this decomposition is associated with the activated water molecule $(\text{H}_2\text{O})'_{\text{act.}}$. An experimental difficulty with which one is faced is that of obtaining the water sufficiently free from impurities. Our attention has particularly been directed to the presence of organic impurities which are easy to detect, because irradiation of gas-free water containing organic material results in the decomposition of the latter, with the formation of hydrogen and carbon dioxide. If one irradiates gas-free water from an ordinary tin still, one may readily find as much as 10 or 20 micromols of these two gases per 1000 cc. For purification of the water, it is subjected to a prolonged heating with acid bichromate and basic permanganate, and a final distillation through a quartz tube heated to 900°C .; by these means it is possible to obtain water which gives on irradiation only 1 or 2 micromols of the two gases. As a final step the water is further purified by prolonged irradiation with X-rays, after which the irradiation test shows less than 1 micromol of hydrogen and carbon dioxide. The slight trace of organic material indicated by the test may be due to the handling of the water as it is transferred to the irradiation cells. We use cells of pyrex glass and, just before being used, possible traces of organic materials are removed by heating the cells nearly to their softening point. After being filled with the solution they are sealed off. Using all of these precautions, we have found in preliminary experiments⁽¹⁷⁾ that there is no change in the decomposition of the water for concentrations of the iodide ion down to 10 micro-equivalents per 1000 cc., while the decomposition is greatly decreased when the concentration is decreased to 0.1 micro-equivalents per 1000 cc. If we interpret these results on the basis of a limited life of $(\text{H}_2\text{O})'_{\text{act.}}$ it may be concluded that the life is at least of the order of 10^{-5} sec. This is a very long life and brings up the question of the nature of these X-ray activated water molecules.

We expected, when we started our work on this subject, that X-rays would produce a dissociation of the water molecules, but the finding

that water is not decomposed by X-rays, as well as the closer study of the chemical properties of the X-ray activated water molecule, leave doubt as to the truth of this. If the X-rays did produce dissociation, then the length of life of the activated water molecule, as estimated above, would be the time for recombination of the dissociated parts to take place, and should therefore depend on the X-ray intensity. This gives a means for experimentally testing whether dissociation occurs. It may be noted that the water molecule present in the vapour form can be activated by collisions with mercury atoms activated by $\lambda = 2537 \text{ \AA}$ ⁽¹⁸⁾. Hydrogen atoms are produced, indicating that the water molecule decomposes into the hydroxyl radical and a hydrogen atom. This type of dissociation has also been observed⁽¹⁶⁾ by heating water vapor to high temperatures, no indication of its occurrence, however, being seen in the absorption spectrum of water.

Water has a strong absorption band for light, which begins around 1760 \AA . We have recently started an investigation⁽²⁸⁾ in which the experiments with X-rays were repeated with light of wavelengths inside this absorption band. The light source was an aluminum spark in hydrogen, and the irradiated solutions were contained in cells of very thin quartz. Preliminary experiments indicate that all the reactions which are attributed to $(\text{H}_2\text{O})''_{\text{act.}}$ can be obtained, but none of those attributed to $(\text{H}_2\text{O})'_{\text{act.}}$. For example, we do not obtain the oxidation of the nitrite ion in gas free solutions, nor the transformation of oxygen to hydrogen peroxide, while the formic acid is decomposed, and the ferrous ion, in the presence of oxygen, is oxidized to the ferric ion. These experiments are carried out with such low concentrations of the reacting molecules, (down to 1 micromol/1000 cc.), that the direct absorption of the light energy by these molecules is negligible. No decomposition is observed when the water itself is irradiated. This is in contradiction to results of a number of investigators⁽¹⁰⁾ (11) (12) (13) (14) who claim to have obtained decomposition of water by the light from a quartz mercury lamp.

Water has another absorption band at 1360 \AA . We hope later to be able to extend our investigations down to this band. Possibly some of the reactions which were obtained with X-rays, but not with $\lambda = 1760 \text{ \AA}$, may be produced with this band of shorter wavelengths.

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DISCUSSION

Dr. French: Have you ever studied reactions in which the irradiation produces oxygen?

Dr. Fricke: The irradiation of the nitrate ion causes its transformation to the nitrite ion with the production of oxygen or hydrogen peroxide, according to the hydrogen ion concentration.

Dr. Hart: Hydrogen peroxide or oxygen is also liberated in the case of irradiation of potassium ferricyanide and potassium iodate, their production depending upon the hydrogen ion concentration of the solution. In acid solution hydrogen peroxide is invariably found, while in alkaline solution hydrogen peroxide appears to be initially formed and then the production of oxygen occurs in a secondary reaction with the potassium ferricyanide or potassium iodate. Thus twice the equivalent amount of oxygen would be expected in alkaline solutions, and this is actually found.

Dr. French: It is your idea that hydrogen peroxide is always the product primarily formed, but that in basic solution it is decomposed with the production of oxygen?

Dr. Fricke: That seems likely.

Dr. Noyes: Hasn't someone shown recently that when one illuminates water vapor with the 1760 Å. radiation, one gets the OH band in fluorescence? Isn't it also true that when one passes a discharge through water vapor one gets fairly large amounts of hydrogen peroxide?

Dr. Fricke: It does not appear likely that hydrogen atoms, oxygen atoms or hydroxyl radicals could be formed in their free state by irradiation of the water by X-rays, since neither oxygen nor hydrogen is produced.

Dr. Noyes: Do you have liquid mercury in contact with the water?

Dr. Fricke: After the irradiation but not before.

Dr. Rollefson: Liquid water molecules are supposed to be at least double, if not more complex. It seems to me that there would be a possibility of such water molecules breaking up on activation in a manner quite different from that in the gaseous state. As a simple example we might assume that a double molecule splits out hydrogen leaving hydrogen peroxide.

Dr. Kassel: I wonder if it would be possible to argue from experiments with platinum electrodes in electrolytic cells that hydrogen atoms do not very readily recombine in aqueous solution. Then the OH radicals produced in the X-radiation of water may form H_2O_2 , but the hydrogen atoms will recombine very little, and mostly react with the hydrogen peroxide, regenerating water. If this is the case, then pure water, although H and OH were formed, would not be expected to decompose.

Dr. Mestre: Is it not possible that the catalytic action of the iodide is simply due to its high mass absorption coefficient relative to that of water?

Dr. Fricke: No, the absorption of the iodide is inappreciable in the low concentration used. However, with concentrations over 1 milli-equivalent per litre an increased rate of decomposition, due to increased absorption by the iodide ion, is observed.

Dr. French: Are biological effects of X-rays due to the activation of water?

Dr. Fricke: It seems quite likely that this may be true in part. However, direct activation of the organic molecules undoubtedly is important too.

Dr. French: Is the absorption of biological materials much different from that of water?

Dr. Fricke: No, not if you make observations on large volumes of biological material.

Dr. Mestre: I have a feeling that perhaps some of the more specific biological effects of X-rays may be due to primary absorption by atoms of very high mass absorption coefficient forming part of molecules of critical biological importance, particularly in the nucleus. A good deal of work has been done tending to show that the distribution of heavy metals is very unequal both in respect to the various organs of the animal body and in respect to cytoplasmic structures.

Dr. Fricke: The influence of a heavy atom is determined by its X-ray absorption relative to that of the matter present in a sphere around it, the radius of which is the length of path of the photoelectron. The influence of the heavy atom would, therefore, be smaller for harder rays, but perhaps may be appreciable for soft rays. The ratio of the coefficients of absorption of two chemical elements depends on the wavelength, which would be reflected in a wavelength dependence of biological effects if the heavy atoms played a role. There is, theoretically at least, a possibility for experimentally testing this notion.

Dr. Marshak: I have made determinations of the absorption coefficients of various biological materials using the $K\alpha$ line of molybdenum. The absorption coefficient of the sperm of *Urechis* and sperm of the cod is 20 to 25% greater than that of water. Now it is true that with harder radiation the difference tends to decrease; for example, with the $K\beta$ line the difference is only about 17%. Still in dealing with masses of material, as one sometimes has to do in biological work, we must also consider secondary radiation, so that even with hard X-rays we may get an appreciable percentage of secondary soft radiation. The biological effect may, therefore, be different from what is obtained from any calculation made on the basis of the absorption coefficient of water; and it seems to me that this is probably the case. Desjardin has published lists of tissues in the order of their sensitivity to X-rays, and it appears that in general the order of sensitivities is proportional to the number of nuclei per unit volume of tissue. The sperm measurements show that the chromatin absorbs more radiation than the rest of the cell. Therefore, those tissues having a greater proportion of nuclei per unit volume are more sensitive. This correlation seems to indicate that the point raised concerning different absorption coefficients is rather an important one, biologically. Apparently the chromatin contains a greater percentage of heavy elements than the cytoplasm or nucleoplasm.

Dr. Cole: If absorption of heavy atoms plays an important role, it should be demonstrable on uniform material by the paired filter method of Ross Kirkpatrick. Two filters are used which have identical spectral absorptions except in a very restricted region where one filter has many times the absorption of the other. When this region includes an absorption limit of the metal in question, there should be a marked difference between the biological effects produced by the two filters.

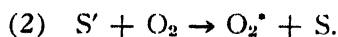
The micro-incineration technique has shown a high salt content in the nucleus and particularly

in the chromosomes. The resultant higher absorption may be the basis of the correlation between sensitivity and the relative amount of nuclear material.

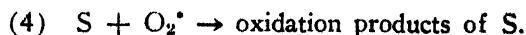
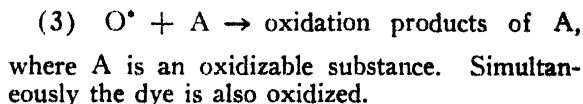
Dr. Marshak: The effect of Alpha particles biologically becomes practically the same as the X-ray effects. There is another point which I suppose should be brought up in connection with mineral matter in tissue. In bone, for example, there is quite a large percentage of calcium. But bone tumors are known to be much less sensitive to radiation. But in that case we have a slightly different situation. There the heavy elements in the intercellular material are effective screens surrounding the nucleus. On the other hand, in the case of blood vessels compared with lymph vessels, we find that the endothelium of the former is more sensitive to radiation. There, you see, the iron of the haemoglobin would act as a radiator of secondary rays, thus increasing the total number of photoelectrons produced in the endothelium.

Dr. Fricke: I think it would be desirable to look into this matter from a quantitative point of view. If this were done, at least some of the effects you consider would be inappreciable.

Dr. Blum: I would like to inject another topic at this time because I would like to have some comments on a reaction which I am studying—possibly involving H_2O_2 . I want to represent schematically this reaction which is the photo-oxidation of substances, particularly biological systems, by light of wavelength around 5000 Å, when sensitizers are present; the sensitizers being usually the fluorescein dyes. The reaction I would like to represent as follows:



This is entirely a schematic representation. These reactions all involve O_2 , and I like to represent them in this way to indicate that in some way oxygen is, let us say, not activated, but is made to take part in other reactions. Now further reactions may be represented:



Now, of course, we can make a good many hypotheses as to what the intermediate steps are. We can assume a formation of a sensitizer peroxide, but there is no real evidence that this is formed, and there is evidence against it. An interesting thing in this connection reported by

Weigert and confirmed by Spealman and myself, is that this reaction is inhibited by low partial pressures of oxygen, and also in high partial pressure of oxygen—the reaction going at the greatest rate in air. This I believe might be evidence against the formation of a sensitizer peroxide. What is particularly interesting, biologically, is the possibility of formation of hydrogen peroxide as an intermediate. We have evidence that a peroxide is formed if we irradiate the dye alone in aqueous solution, and this seems to be hydrogen peroxide. Remember that the dye is always oxidized in this process and this gives the possibility of numerous reactions. The best evidence that hydrogen peroxide is formed comes from the study of biological systems. In a simple, non-biological, system, containing an oxidizable substance, if we introduce cyanide we get no effect on the rate of the reaction. In a biological system we get augmentation of the reaction when we introduce cyanide. We can explain this if we assume that the catalase which is present in most biological systems, and which catalyzes the decomposition of hydrogen peroxide, ordinarily keeps the rate of the oxidation process down, the introduction of cyanide will inhibit the catalase and augment the oxidation. The specificity of catalase for hydrogen peroxide seems to be quite definite, at least catalase has never been demonstrated to catalyze the decomposition of any organic peroxide or peroxy-acid. I present this schematic arrangement for your consideration. I would like to introduce probable intermediate steps, but I have not felt it wise to do so.

Dr. Fricke: Is there any spectral limit to the reaction?

Dr. Blum: The primary reaction is the activation of the sensitizer and all the sensitizers available absorb below about 5500 Å., so that the reactions are limited by the specific absorption, if not by anything else. The reaction $2\text{H}_2\text{O} + \text{O}_2 \rightarrow 2\text{H}_2\text{O}_2$; $\Delta H = +46,000$ cal. is possible up to 6200 Å., which includes the absorption spectra of all the dyes we have used.

Dr. Fricke: Have you tried to use sensitizers absorbing in wavelength regions longer than 6100 Å?

Dr. Blum: We would like to do that but have not found a suitable system.

Dr. Noyes: Do you have any idea of what the quantum yield is?

Dr. Blum: The only figure we have is for thiosulphate which is not a good system to use, it is very low, about 0.035, which is only a tentative value.

Dr. Rollefson: I think that it is probable that a peroxide is formed by reaction between the activated state produced by light and the oxygen rather than that the activation energy is transferred to the oxygen.

Dr. Fricke: Is the absorption spectrum of the dye dependent on the presence of oxygen?

Dr. Blum: No. Another point which may be of interest to you: hydrogen peroxide will not bleach the dye in the absence of light in neutral solution but if we add hydrogen peroxide to a solution of the dye and then irradiate, the rate of bleaching is greater than when no H_2O_2 is added. This has lead me to the suggestion that the first step in bleaching is the formation of H_2O_2 , resulting in the oxidative bleaching of the dye molecule.

Dr. Fricke: Are you working outside the absorption spectrum of H_2O_2 ?

D. Blum: Yes.

Dr. Noyes: In what state is the dye, true solution or colloid?

Dr. Blum: True solution.

Dr. Fricke: The reactions produced with hydrogen peroxide depend on the hydrogen ion concentration. It would, therefore, be of interest to carry out your studies at different values of the pH.

Dr. Blum: The effects apparently decrease with increasing hydrogen ion concentration but I am not sure of this point.

Dr. Noyes: It is always suspected in most bleaching dyes in textiles—a subject which comes up in New England whenever talking to a textile chemist—that adsorbed oxygen is important.

THEORY OF CATALYSIS IN SURFACE REACTIONS OF PHOTOCHEMISTRY

HUGH S. TAYLOR

There are relatively few investigated surface reactions of photochemistry but they are of great importance. They include the reactions of the photographic plate, photosynthesis and phototropism in biological systems. There is no one theoretical treatment generally applicable. Each system requires individual consideration and presents features which may, however, be common to several such reaction systems. We cannot, therefore, present any single generalised theoretical treatment but must content ourselves with an illustrative discussion of typical mechanisms in which catalytic features are exhibited.

Catalysis intrudes in surface reactions of photochemistry generally in the form of photosensitisation. By this we mean the securing of a photoreaction in a system of reactants which do not absorb the light employed, by incorporating in the system a sensitizer which absorbs the light energy and in some manner transmits such absorbed energy to the reactants, the sensitizer itself normally remaining unchanged on completion of the process. The sensitisation of gelatine-silver halide emulsions, by means of adsorbed dyestuffs, to light of long wave lengths, is one of the earliest and best known of such sensitisation processes and it involves a surface reaction. Such a system can be used as typical of sensitisation in processes of photodecomposition. It will, however, also be shown that sensitisation at surfaces governs the processes of assimilation of carbon dioxide in plant life and of oxidation in various processes likewise of biological significance.

Light Absorption by Sensitizer: The primary absorption act in such sensitised processes raises the absorbing molecule (or atom) to a higher energy level from which it may recede either by light emission or by transfer of this energy to systems in contact with the energy-rich unit. In atomic sensitizers the problem is simpler, since there are fewer possibilities of light absorption and emission. The emission in such cases is normally of the same wave length as that of the absorbed light, although multiple absorption, prior to emission, may change this. In molecular sensitizers the emission is evident as some type of fluorescence and, in sensitisation processes generally, the sensitizers can be shown to fluoresce. The relation between absorption and emission in molecular systems is not simple. A variety of wavelengths absorbed may produce the same type of fluorescent emission.

Collision or contact with other molecules by an excited sensitizer suppresses the fluorescence, the energy which would have been lost in the

emission process being received by the "acceptor" in collision or contact. The quenching of fluorescence in an absorbing agent may be taken as a certain index of energy transfer to an "acceptor", and may often be shown to be the prelude to the subsequent photochange. Such quenching has been thoroughly studied in atomic gaseous systems and its main features have been competently analysed¹. In heterogeneous systems the phenomena are more complex. Special experiments must be devised to ascertain by what constituents of the system the quenching of the fluorescence is achieved. This will vary from system to system.

Secondary Processes: In the sensitised gelatine-silver bromide emulsion the simplest assumption is that the energy is given up by the sensitizer to the silver halide particle in the immediate neighborhood, and this particle is decomposed. The primary process of reaction is assumed to be a dissociation of the silver halide to a silver and a halogen atom. In the sensitisation process, the sensitivity maximum is not co-incident with the maximum of absorption of the sensitizer dye, but is generally displaced to the red. This points to a dye-halide compound or adsorption complex as a photo-active ensemble. It is known, however, that the dye stuff need not be destroyed in the reaction occurring, and a single dye molecule may sensitise the decomposition of many halide molecules.

It is also known that another type of catalysis operates in the photographic process. The light sensitivity of the silver halide grains is dependent on the purity of the grain and also on the presence of the gelatine. This catalysis is due to the functioning of the gelatine or impurities as acceptors for the halogen atoms produced. This is evident from a study of quantum yield in the photolyses of solid silver halides. With compact silver bromide the yield is less than 0.01. Moist precipitated silver bromide shows quantum yields in the neighborhood of 0.1. With gelatine silver bromide emulsions the yield rises to unity. It is evident that the gelatine is a receptor for the bromine atoms, preventing recombination of the silver and halogen atoms. Adsorbed materials operate similarly in the moist precipitated halides, but less efficiently. In the latter case, also, there is a true photosensitisation, since adsorbed silver nitrate has been shown to heighten the light sensitivity; the precipitate is made more red sensitive, the displacement of light absorption being ascribed to strong deformation of the bromine ions in the surface by the adsorbed silver ions.

Neutral silver atoms also can act as sensitisers, as revealed by the Becquerel-effect. Becquerel showed that halides previously illuminated by blue light are made more sensitive thereby to green light. It is the photochemically produced silver atoms which bring about this sensitisation.

In the processes of photosynthesis, it is the chlorophyll which acts as photosensitiser. It is known that chlorophyll fluoresces intensively in the red, and that this fluorescence is strongly quenched by oxygen. Fluorescence is demonstrable even in living green leaves and the intensity relations are quite characteristic, as recently demonstrated by Kautsky². At the outset of illumination there is a strong variation in fluorescence, first a sharp rise to a maximum, then a slow decrease to a final constant stationary state of fluorescence. Kautsky has shown that, in living green leaves, completely freed from "free" oxygen, even in presence of moisture and carbon dioxide, the chlorophyll begins to fluoresce with high intensity, without any initial increase of intensity. This indicates that the changes in fluorescent intensity are dependent on the concentration of oxygen in the chloroplasts and that normal oxygen is the molecule to which the energy absorbed by the sensitiser is transferred. The oxygen in the chloroplasts, present in equilibrium with that of the surrounding atmosphere, is responsible for the initial quenching of fluorescence; during the initial rise in intensity this oxygen is consumed, and the period of decreasing intensity is accounted for by regeneration of oxygen in the assimilation process. Kautsky showed that other gases like nitrogen, hydrogen, and especially carbon dioxide, as well as many organic substances were not able to quench the chlorophyll fluorescence. Furthermore, poisons for the assimilation process, such as hydrogen cyanide, have no influence on the initial rise of fluorescent intensity to a maximum, but prevent the subsequent decrease in intensity of fluorescence, since with the assimilation process poisoned no oxygen is regenerated.

Kautsky also showed in special experiments that the intensity of fluorescence in sediments of chloroplasts was conditioned by the available oxygen and that access of this gas to the chloroplast was determined by the pH of the medium. With alkaline sediments the velocity of diffusion of oxygen increases more rapidly than the oxygen consumption in the interior of the chloroplasts. All of the experiments lead to the conclusion that the oxygen is the only molecule in the assimilation system to which the energy absorbed by the chlorophyll is conveyed. This case is sharply differentiated from the photographic sensitisation process. A gas, oxygen, acts as an intermediate, be-

tween sensitiser and reactants, as collector and carrier of the assimilation energy.

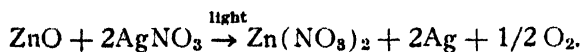
In the photosynthetic processes the energy-yielding reaction of the active oxygen molecules with constituents of the chloroplast is evidently coupled in some way or other with the energy-consuming processes of assimilation, and the oxygen is ultimately regenerated. It is evident that the same kind of sensitised oxygen activation by chlorophyll can give rise to true processes of photosensitised oxidation. Examples of such with various sensitisers have also been found by Kautsky and his coworkers³. They have shown that, in spite of a separation in space of sensitiser and acceptor, oxidation of the latter may occur when oxygen is present, the experiments revealing that the phenomenon occurs only within well defined pressure limits dependent on the spatial separation of acceptor and sensitiser. At too low pressures the rate of active oxygen production is too small. At too high pressures the active oxygen, being metastable, is destroyed before it reaches the acceptor to produce oxidation. This concept of Kautsky's as to the inner mechanism of a whole group of photosensitised oxidation processes is in sharp contrast to earlier ideas. More generally it has been assumed that the energy absorbed by the sensitiser was transmitted to the acceptor directly, bringing it to an activated state in which it could readily react with normal oxygen. In his recent work, Kautsky definitely disposes of such a mechanism in a group of photosensitized oxidations by demonstrating, in special phosphorescence experiments, that the oxidisable compound was completely incapable of quenching the phosphorescence observable after illumination, whereas oxygen even in small traces was very efficient as the quenching agent. Kautsky identifies the active oxygen so produced as the $^1\Sigma$ term of the oxygen molecule with an energy equivalent to approximately 38,000 calories. Spectroscopic evidence indicates that it is a metastable state of the molecule with a lifetime of several seconds. Since, in addition to strongly fluorescing dyestuffs such as tryptaflavin, biologically important coloring matters such as haematoporphyrin and chlorophyll possess the characteristics that have been discussed, it is evident that this mechanism of oxygen activation has fundamental importance in the field of biological photochemistry.

An interesting set of reactions catalytically accelerated by light centre around zinc oxide as sensitiser. It has long been known that oil paints containing zinc oxide as pigment deteriorate relatively very rapidly in sunlight. Lithopone paints are also markedly light sensitive and in this case the sensitivity is markedly influenced by impuri-

ties in the pigment. Such zinc preparations may also be shown to display marked fluorescent phosphorescent effects.

The action of zinc oxide as sensitizer has been studied by numerous investigators, including Winther⁴, Baur and Perret⁵, and latterly by Jung and Kunan⁶ and by McMorris and Dickinson⁷. There is as yet no completely satisfactory theoretical treatment of the experimental material. The most extraordinary of the photosensitisations using zinc oxide studied by Winther, the ozonisation of oxygen, is now known not to occur. It has now definitely been shown that the product is not ozone but nitrogen peroxide, liberated from an incompletely ignited zinc oxide prepared from the nitrate. Ozonisation of oxygen would have been very extraordinary owing to the wave length of light required to produce this product from oxygen. Since, in the experiments, only long wavelength ultraviolet was used for excitation, the formation of ozone would have suggested a conversion of such exciting light into a much more energy-rich short-wave ultraviolet fluorescence. There are no well-investigated cases of such considerable "anti-Stokes" fluorescences.

Illuminated zinc oxide reacts with silver nitrate in solution, depositing silver and some silver peroxide and also forming zinc nitrate and producing evolution of oxygen. Stoichiometrically the reaction may be expressed thus:



For each atom of silver liberated, $1/4 \text{O}_2$ is formed either as free oxygen or peroxide. In a similar way illuminated zinc oxide reacts with solutions of mercuric chloride, producing mercurous chloride and likewise liberating oxygen.

These oxygen producing reactions can be coupled with oxidation processes, which may also be accelerated in presence of zinc oxide when oxygen is present. The oxidation of glycerine and of various dyestuffs has been secured in this manner. Perret⁸ showed that even in the absence of inorganic salts and oxygen gas, zinc oxide on illumination effects the reduction of methylene blue to the leucobase, a portion of the dyestuff being simultaneously oxidised. The presence of other oxidisable materials, such as glucose, reduces the amount of dyestuff so oxidised. In all of these photosensitive reactions involving solid zinc oxide there is no definite theoretical treatment possible. Even the primary light process is not known as to nature, and the coupled reactions are also complex. The reactions may well repay study since they bear some resemblance to biologically interesting photosensitive systems.

Surface Catalytic Effects Associated with Chain Reactions: As is now well known, a number of reactions are known in which the primary light process is succeeded by a long chain of secondary processes so that the equivalence between light energy absorbed and chemical reaction occurring no longer obtains. It is evident that if, in such chain mechanisms, the initiator of a chain can be produced photochemically, pronounced catalytic effects may result on illumination.

The oldest known process of this type is the decomposition of hydrogen peroxide solutions, studied by W. Kistiakowsky⁹, produced by illumination of solutions of potassium ferro- and ferricyanides. The mechanism in this case is well understood. The light action produces a colloidal decomposition product of the iron cyanide complexes. At these surfaces, photochemically formed, the peroxide decomposition chain is initiated. As suggested by Haber and Willstätter¹⁰, the decomposition of a peroxide molecule at the colloidal surface involves a change in the valency of the iron, and radicals may be liberated into the solution, there initiating a long chain of secondary decompositions. The enzymic action of catalase in peroxide solutions is also attributed by them to such a chain initiation, though experimental evidence to demonstrate this convincingly is lacking. In principle, however, it is evident that if a process of light absorption at a solid surface may be utilized to liberate a chain initiator, in the form of an atom or a radical, into a solution in which such secondary chain processes can readily occur, there are present all the conditions necessary for an important surface photosensitive catalytic process. The work of Rice¹¹, indicating the importance of dust particles in the photodecomposition of hydrogen peroxide solutions, may well find its explanation in a phenomenon of this kind. Similar effects might also be found in other known, chain-mechanism, decomposition and oxidation processes.

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DISCUSSION

Dr. Brackett: I have in mind observations wherein Dr. Karl Meyer found that in a system where chlorophyll is introduced into ergosterol solution in cyclohexanol, there is biological activation of ergosterol, in which each mole of ergosterol takes up half a mole of oxygen in the presence of visible radiation.

Dr. Kistiakowsky: In what sense do you mean activation?

Dr. Brackett: Antirachitic activation as shown biologically. It is interesting that with certain other dyes he found that there is a full mole of oxygen taken up and no biological activation.

Dr. Fricke: Is oxygen also concerned in the activation of ergosterol by ultraviolet radiation within its own region of spectral absorption?

Dr. Brackett: I think not.

Dr. Kistiakowsky: I would like to say that it seems to me that this photo-action is of extreme importance and could be subjected to direct experiment. There is only one state of the oxygen molecule at an energy level, consistent with activation by visible radiation, and that is the $^1\Sigma$ state which is also responsible for the atmospheric bands. It is true, of course, that absorption of light there is extremely weak. It takes a very long layer of oxygen to produce marked absorption, but the absorption is not zero, and it might be possible by using very high intensity of red light and oxygen at high pressure to test the activity of the $^1\Sigma$ state directly. It seems to be of some interest and importance, and is certainly worth considerable effort. It is possible to produce red light of such high intensity that the experiment is not quite hopeless.

Dr. Noyes: The transition from the normal $^3\Sigma$ to the $^1\Sigma$ state has a low probability, first because of the change of multiplicity rule and second because it is probably a plus-minus transition instead of a plus-plus. I am wondering if these selection rules hold in case the oxygen is in some medium other than in the gas phase. They may break down in the presence of the field of other molecules.

Dr. Kistiakowsky: I think that the change-of-multiplicity rule would be more likely to break down than the plus-minus rule.

Dr. Noyes: Would you expect $^1\Sigma$ oxygen to be more reactive than the normal molecule?

Dr. Kistiakowsky: I think that question is answered by Kautsky's work.

Dr. Noyes: I wouldn't say that it proved that a $^1\Sigma$ state of the molecule is involved.

Dr. Kistiakowsky: I imagine that unless you reinterpret his entire work, you really have to accept the $^1\Sigma$ molecule.

Dr. Noyes: Mullikan is of the opinion that in the oxygen molecule there are states which have not been found spectroscopically such as a $^3\Delta$ state, and I would prefer to use one of them rather than the $^1\Sigma$.

Dr. Kistiakowsky: The $^1\Sigma$ is probably less reactive.

Dr. Noyes: I believe that the heat capacity evidence for these other states is not reliable, but they probably do exist.

Dr. Mestre: If Kautsky's interpretation of the mechanism of photosensitized oxidations is substantiated, it may, as Taylor has pointed out, well have fundamental importance in the field of biological photochemistry. There would, however, seem to be a number of objections which might be raised against the acceptance of Kautsky's extension of his activated O_2 mechanism to include the photochemical reduction of CO_2 in the plant. Among these I might mention the extremely low fluorescence yield in the leaf even in the absence of oxygen. It would seem as though the fluorescence efficiency should be very much higher under these conditions if oxygen is the sole acceptor of energy from the chlorophyll as postulated by Kautsky. As my other comments relate rather to Kautsky's work than to Taylor's paper, and as I intend to discuss these in my paper on the photosynthetic system of the chromatophore (later in this volume), I would prefer to reserve them until that time.

Dr. Brackett: Two objections to the mechanism proposed by Kautsky as an explanation of his observations regarding the quenching of chlorophyll fluorescence by O_2 have been offered:

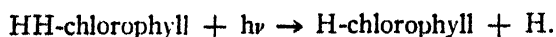
1) This mechanism requires that O_2 be the energy acceptor in a collision of the second kind with accompanying electronic excitation of O_2 to a low level. Since there is no evidence that such an excited O_2 molecule would be particularly reactive, this offers little help in understanding the mechanism of photosynthesis.

2) As Mestre points out, Kautsky's mechanism does not explain the relatively low fluorescence yield in the absence of O_2 .

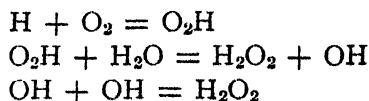
These difficulties, together with other objections, have led J. Franck to propose an entirely different mechanism. His explanation, extending to the larger problem of CO_2 assimilation, has been presented in an article published in *Naturwissenschaften* 14: 230, 1935. Franck's mechanism is based upon the observations of Stoll regarding the existence of two H atoms loosely bound to chlorophyll, and closely parallels the reaction scheme proposed by Willstätter.

Starting with a fully hydrogenated chlorophyll, indicated by "HH-chlorophyll", the absorption of one quantum results in a partial dehydrogenation of chlorophyll to monodehydrochlorophyll, "H-

chlorophyll", with the production of a free hydrogen atom:



This is followed by the reactions proposed by Haber:

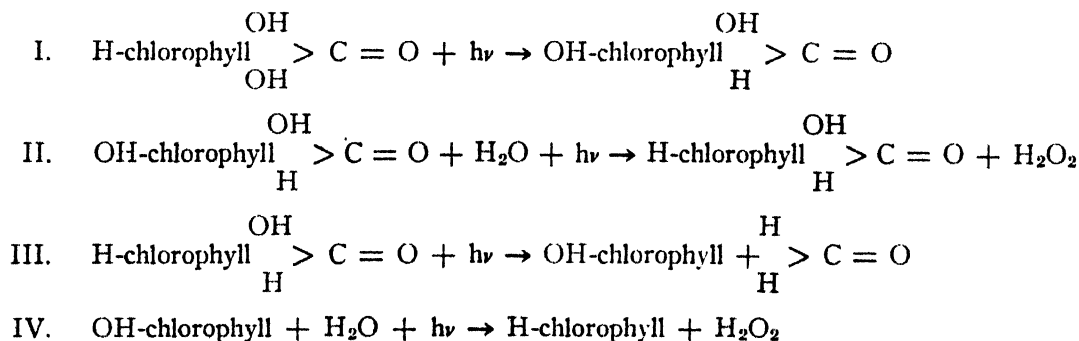


This series of reactions results in an induction period before actual assimilation begins. In the absence of O_2 , H combines with monodehydrochlorophyll, resulting in chemiluminescence and the regeneration of fully hydrogenated chlorophyll. The presence of O_2 , on the other hand, provides for the removal of the H atom, thereby preventing chemiluminescence. This accounts for the observations of Kautsky without requiring a metastable excited chlorophyll state. In the presence of CO_2 the first step of real assimilation begins. Assimilation is then cared for in a stepwise process as follows:

points out that the preliminary partial dehydrogenation of HH-chlorophyll may proceed with relatively low efficiency as would be expected from general considerations without interfering with a high quantum efficiency in the real assimilation. This is in accordance with the relatively low fluorescent yield observed and adequately explains Kautsky's observations.

Franck accounts for the energy requirements of the steps on the basis that OH is half as tightly bound to dehydrochlorophyll as is H. Consequently in steps II and IV he can credit the reaction for this exchange with 21 k. cal. Furthermore, he assumes that the formation of H_2O_2 from 2 OH represents 52 k. cal. Then with the 41.4 k. cal. furnished by the absorption of a light quantum the entire 115 k. cal. which he assigns to the dissociation of water into H and OH is accounted for. If, on the other hand, the OH is less than half as tightly bound as the H, steps II and IV proceed with an excess of energy.

While step I requires only a rearrangement, step III requires the separation of formaldehyde from OH-chlorophyll as well. Franck does not



In all these steps involving energy uptake, it is assumed that the energy provided corresponds to the wavelength limit of fluorescence, 6800A, i.e., a binding energy corresponding to 1.8 volts or 41.4 k. cal. In every case the energy requirement is presumably less than that available. Franck

discuss the energy requirements of these steps.

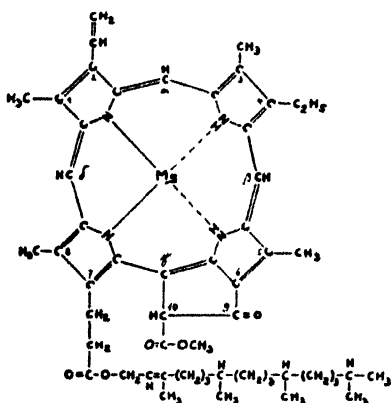
At any rate, Franck has offered an extremely interesting explanation of Kautsky's observations, and a mechanism for photosynthesis which avoids the difficulties introduced by the assumption of a metastable state in chlorophyll.

PROTOCHLOROPHYLL

PAUL ROTHMUND

Although the greening of plants has been studied for many years, the problem has not been solved. Investigations on the formation of chlorophyll from the chemical point of view will probably also contribute much to the elucidation of the physiological role of the green pigment in nature. It is known that chlorophyll formation in most plants represents a photochemical process; plants grown in the dark—etiolated plants—usually do not form chlorophyll. They contain, however, a certain amount of yellow pigments, and a very small quantity of a green substance. The latter exhibits red fluorescence in its solutions and differs spectroscopically from chlorophyll. Most of this green pigment disappears upon irradiation of the plant and is then apparently transformed into chlorophyll.

Chlorophyll occurs in the green plant in two forms: chlorophyll *a*, $C_{55}H_{72}N_4O_5Mg$, and chlorophyll *b*, $C_{55}H_{70}N_4O_5Mg$, in most plants in the ratio $a : b = 3 : 1$. From his extensive studies of chlorophyll Fischer (1) derives the following formula as the most probable expression for our present knowledge of the structure

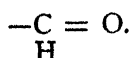


Chlorophyll *a*.

M. Fischer 1935.

FIGURE 1

of the molecule. In chlorophyll *b* the methyl group in position 3 is replaced by the formyl group



The protochlorophyll problem is represented by the question of the mechanism which brings about the formation of the two pigments, chlorophyll *a* and chlorophyll *b*. A number of other physical and chemical conditions, besides the presence of radiant energy, are, of course, necessary for

building up chlorophyll. For details on these various factors the reader is referred to Kostytshew (2) and to a recent study by Inman, Rothmund and Kettering (3).

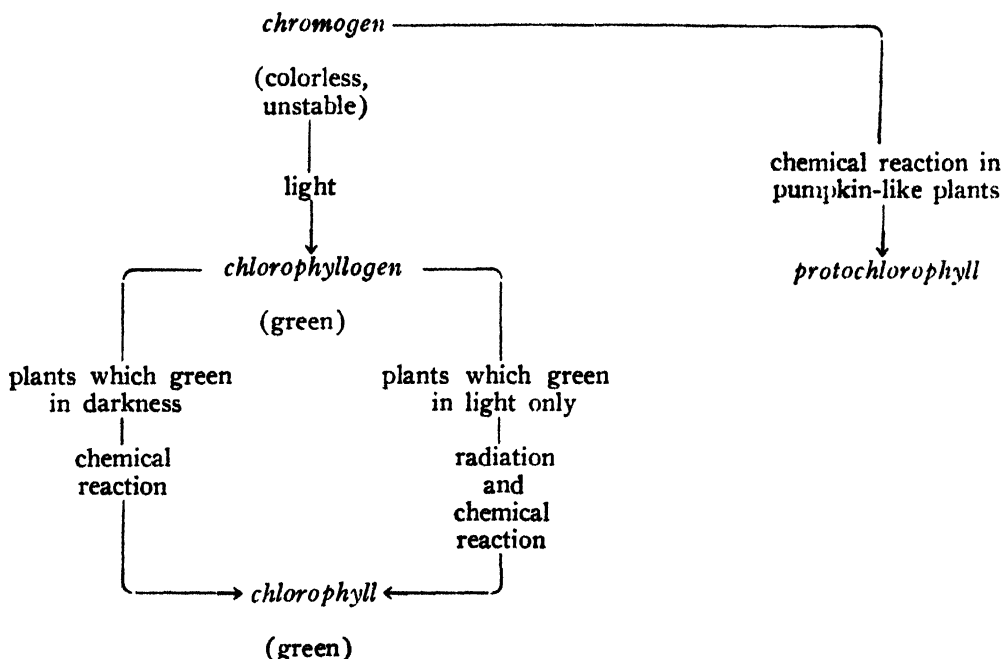
A historical survey will indicate best the different opinions and explanations offered by the investigators in the field to account for the greening of plants.

Almost one hundred years ago Preisser (4) suggested that leaf green—chlorophyll—is formed by oxidation of a colorless compound, a particular chlorophyll-chromogen. This compound was studied very extensively and appears in the literature under different names: colorless chlorophyll (Schleiden), chlorophor (Boehm), leucophyll (Sachs), etiolin (Pringsheim), protophyllin (Timiriacheff), and protochlorophyll (Monteverde).

Pringsheim (5) was the first to report the typical band at 640 - 620 $m\mu$ in the absorption spectrum of etiolin. Although his solutions contained a certain amount of yellow plant pigments, the spectroscopic record indicates the identity of Monteverde's protochlorophyll (6) and etiolin. For historical reasons the substance under discussion should probably be named etiolin. However, in the botanical, and in the chemical, literature the term protochlorophyll is preferred and it is due to this fact that the author of the present paper follows the customary usage. Monteverde extracted protochlorophyll from wheat, maize, and sunflowers by means of 95% alcohol. A good review of the older literature on chlorophyll and chlorophyll-development is found in his article. Czapek (7) discusses the greening process of plants in detail in his book. Greilach (8) obtained protochlorophyll from *Hordeum sativum*, *Lepidium sativum*, *Cucurbita Pepo* and from *Phaseolus multiflorus*. He defined protochlorophyll as the pigment in etiolated plants which produces in alcoholic solution a strong absorption band at 640 - 620 $m\mu$ and a weaker band at 589 - 570 $m\mu$. Greilach believed that under the influence of light the transformation of protochlorophyll into chlorophyll took place. According to Liro (9) and Isatschenko (10), light is only one step in a sequence of reactions, in the course of which the colorless chromogen (= Sachs's leucophyll) is changed into chlorophyll. The last reaction in the sequence is, according to these authors, a strictly chemical one and takes place in dead cells as well as in finely ground cells which are kept in an oxygen-free atmosphere. Leucophyll formation, however, depends upon the presence of oxygen, according to this theory.

The red rays in the spectrum, for which chlorophyll exhibits strongest absorption, are also the most efficient ones to bring about chlorophyll formation, a fact reported by Wiesner (11) and recently confirmed by Sayre (12).

Monteverde and Lubimenko (13) studied the pigments from the seed of pumpkin-like plants and derived a new theory on chlorophyll formation from their experiments. They believe that under the influence of light a very unstable colored substance, "chlorophyllogen", arises out of an unknown colorless chromogen. The chlorophyllogen in turn yields chlorophyll. Protochlorophyll is supposedly formed only by the pumpkin-like plants and in the absence of light. The conclusions of Monteverde and Lubimenko can be given in a diagram of this form:



Schmidt (14) investigated the chlorophyll formation in conifers and came to the following conclusions: Chlorophyll produced in the dark by these plants is spectroscopically identical with chlorophyll formed in light. Extirpation of the colorless embryo from the endosperm causes complete etiolation. If a small amount of endosperm is still connected with the radicle, or if the embryo is brought in contact with endosperm, the seedling will form chlorophyll in the dark. The developmental state of the embryo, or of the endosperm, does not influence the result of the experiment. Dead endosperm, however, is ineffective. The conifer seedlings contain in the endosperm a chlorophyll-building substance which can replace light in the process of chlorophyll formation.

Recently the protochlorophyll problem was studied in detail by Noack (15). The inner green seed coat of Cucurbitaceae was extracted. Noack considered the protochlorophyll thus obtained as being, essentially, a reduced magnesium-containing precursor of chlorophyll, with porphyrin properties. No evidence was found of the existence of two forms which would correspond to chlorophyll *a* and *b*. Irradiation of crude protochlorophyll solutions in alcohol, or acetone, caused the appearance of the typical red band of chlorophyll in the absorption spectrum of the solutions. Noack also prepared a number of protochlorophyll derivatives, and his analytical studies led him to the assumption that protochlorophyll contains three carboxyl groups, two of them being esterified with methanol and phytol, re-

spectively, while the third one is present in lactone or lactam form. A concise review based to a large extent on Noack's measurements and results, is given by Treibs (16). The absorption spectrum of protochlorophyll in the living leaf was demonstrated by Noack and by Scharfnagel (17). On account of this finding, the theory by Liro and by Lubimenko of protochlorophyll being formed upon decomposition of chlorophyllogen in the dark, could not be maintained.

Lubimenko (18) experimented with carefully selected seedlings of a pure strain of *Triticum ferrugineum*; the chlorophyll accumulation in these seedlings at different temperatures was studied and the greening process was represented by the following sequence of changes:

1. Synthesis of leucophyll (Sachs' terminology).

2. Transformation of leucophyll into chlorophyll.

3. Transformation of chlorophyll into chlorophyll.

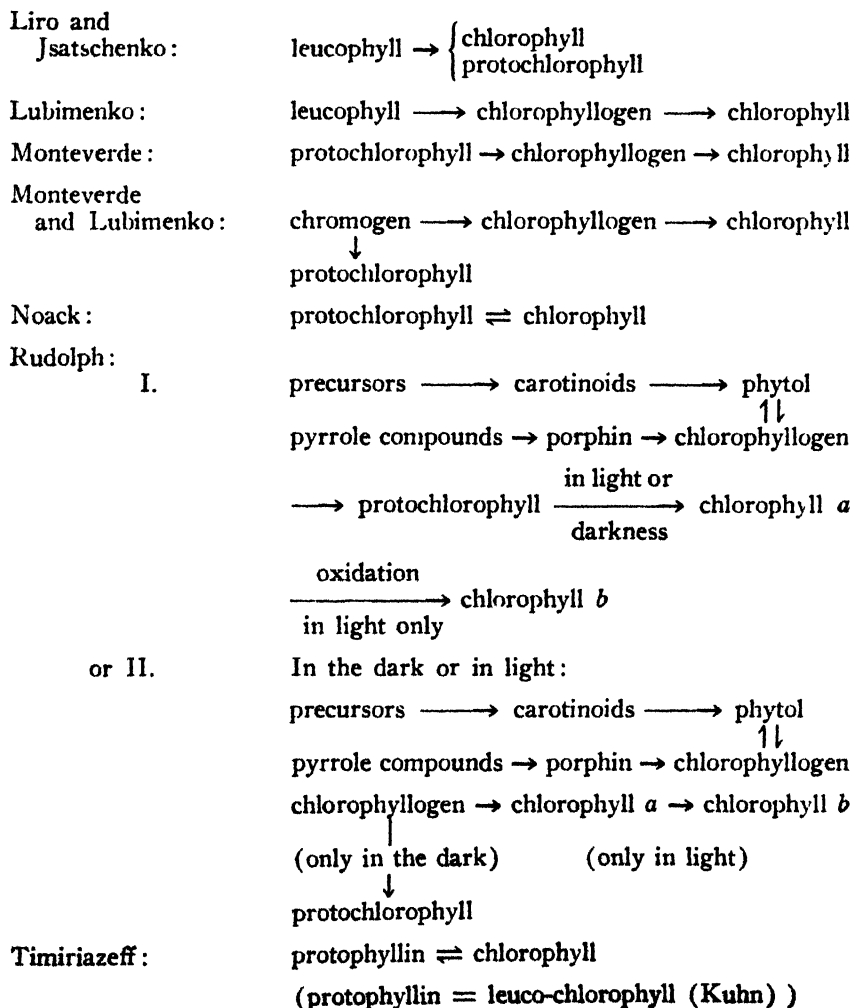
Reactions 1 and 2 occur in the dark, reaction 3 is strictly photochemical. Chlorophyll, appearing in reactions 2 and 3, is a green pigment, standing optically near chlorophyll. According to Lubimenko, chlorophyll is identical with Monteverde's protochlorophyll.

The possible action of carotinoid pigments in chlorophyll formation was recently discussed by Euler (19), by Godnev (20), and by Smith (21).

Rudolph (22) also considered the carotinoids to be involved in the reaction, believing that they furnish the building material for the phytol component of the chlorophyll molecule. For the protochlorophyll of bush beans, Rudolph reported

three maxima in the absorption spectrum, one in the red, one in the yellow-green, and one in the blue region. The position of the first band (625 mμ) is somewhat different from the values reported by other workers for the same band. The relative extinction coefficients of protochlorophyll were determined by Rudolph from $\lambda = 640 \text{ m}\mu$ to $\lambda = 537 \text{ m}\mu$. A relation between the quantity of protochlorophyll and the leaf area could not be found. The pigment did not disappear quantitatively upon irradiation, but its decrease in light was not proportional to the amount of chlorophyll formed. Rudolph demonstrated a distinct relationship between the carotinoids in the plant and chlorophyll formation during the first, and after the fourth, hour of irradiation.

It might be helpful to represent the principal recent theories on the role of protochlorophyll in chlorophyll formation on a chart by names of authors:



The system protochlorophyll \rightleftharpoons chlorophyll has often been considered an oxidation-reduction system, and many authors have studied the behavior of chlorophyll toward reducing agents. Berzelius' method (23) of reducing an alcoholic chlorophyll solution with zinc and hydrochloric acid caused complete destruction of the molecule. Timiriazeff (24) reduced chlorophyll in pyridine with zinc and an organic acid. A colorless solution resulted, lacking entirely the characteristic chlorophyll spectrum. In contact with air, the green color and the specific optical properties of chlorophyll were gradually acquired again. Timiriazeff called the reduced compound colorless chlorophyll or protophyllin.¹

Timiriazeff's method was rediscovered recently by Kuhn (26) who applied it to a number of pigments, including chlorophyll *a* and *b* and some chlorophyll derivatives. Upon air oxidation the chlorophylls were regenerated completely, as demonstrated by positive Molisch reaction ("phase test"), by absorption spectrum and by elementary analysis.

By reduction with iron powder in 80% formic acid, Noack (27) prepared a number of chlorophyll derivatives, which he considered the analogues of chlorophyll in the protochlorophyll series.

Catalytic reduction was used by Fischer (28) and by Stoll (29); the compounds obtained were spectroscopically and chemically different from Timiriazeff's and from Noack's products.

In summarizing this literature survey, it can be stated that the viewpoints of the various authors on protochlorophyll and its role in the synthesis of chlorophyll are very contradictory. Many difficulties are due to the fact that neither protochlorophyll, nor any other precursor of chlorophyll, has been isolated in pure state, as yet.

For the work in our laboratory, two lines of attacking the protochlorophyll problem presented themselves: 1. the extraction, and possibly isolation, of protochlorophyll, or of any precursor of chlorophyll from etiolated plants, 2. the study of reduction products of chlorophyll and its derivatives, and their comparison with substances found in etiolated plants.

The preparation of protochlorophyll extracts from white and yellow corn, grown in absolute darkness, is under way.² Extraction of the plant meal with ether yields a protochlorophyll containing liquid with the first absorption band from 630

to 620 m μ . This value agrees better with Rudolph's measurements than with the values given by Pringsheim, Monteverde and others. A benzene extract of the plant meal does not show well-defined absorption bands. The extract is subjected to chromatographic adsorption (Tswett's method (30)) on aluminum oxide. The sequence of layers, from top to bottom of the chromatogram tube in a somewhat simplified form, is green, yellow, brown, yellow, pink, olive green, orange, yellow. The upper green zone is the relatively broadest one. It is eluted with hot pyridine and the pigment transferred into ether by addition of water. The ether solution from direct extraction of ground etiolated corn, as well as the pyridine or ether solution obtained from the green aluminum oxide adsorbate, is of yellow color with a tint of green and shows red fluorescence.

A substance which, from the method of preparation, might be called protopheophytin results from treating an alcoholic extract of corn meal with a small quantity of acid, or from extracting the etiolated corn with acetic acid and transferring the pigment into ether.

Fig. 2 gives the absorption spectra of these substances and the fluorescence spectrum of protochlorophyll from corn in ether.³ The fluorescence spectrum of protochlorophyll from wheat in methanol, as recorded by Dhéré (31), is inserted in Fig. 2 to illustrate the influence of the solvent on the position of the fluorescence band. The marked difference in the absorption spectra of protochlorophyll and of the green pigment from etiolated corn is clearly visible.

Solutions of protochlorophyll or of the green pigment in acetone, alcohol, ether or pyridine do not turn green upon irradiation; chlorophyll formation out of these pigments *in vitro* does not occur.

² Unpublished experiments with the cooperation of T. Londergan. Corn was chosen because it has a fairly rapid rate of growth and, therefore, renders available relatively large quantities of the starting material in a short time. The corn is collected after two weeks of growth and worked up immediately by heating it in a steam sterilizer for 15 to 20 minutes at 126°C, the temperature which corresponds to the pressure of 20 lbs. per square inch. After this treatment, the corn is no longer sensitive to light. It is dried at a temperature of 30 to 40°C, ground to a fine powder, and extracted. The heating is not considered objectionable because, in orientating experiments, drying and extraction in complete darkness had led to the same products as the faster and more convenient method using the sterilizer.

³ I am indebted to Professors Albers and Knorr for the spectro-photograph of this fluorescence spectrum.

¹ This term may lead to confusion: according to Willstätter's definition for phyllin (25), and in the light of numerous phyllin syntheses by Fischer, the term would be correct for the magnesium complex salt of any of the 15 isomers of protoporphyrin (Tetramethyl-divinyl-dipropionic acid porphyrin).

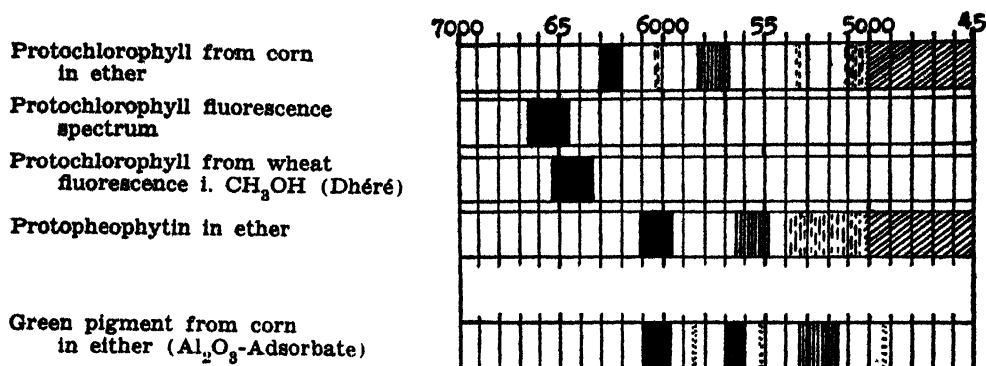


FIGURE 2

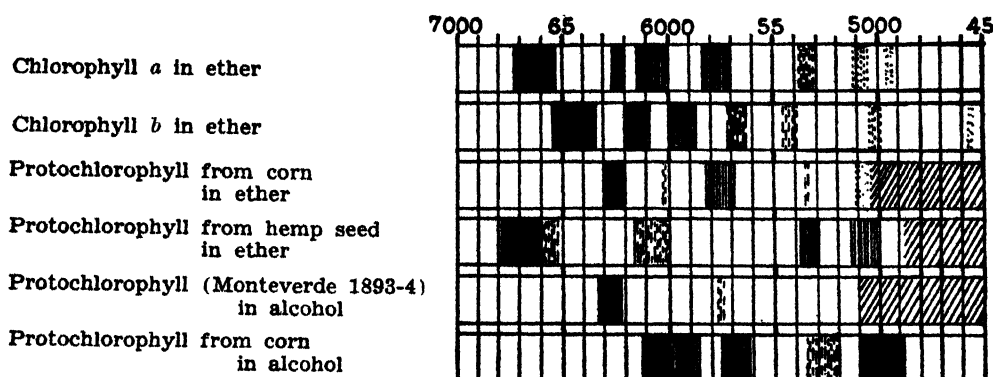


FIGURE 3

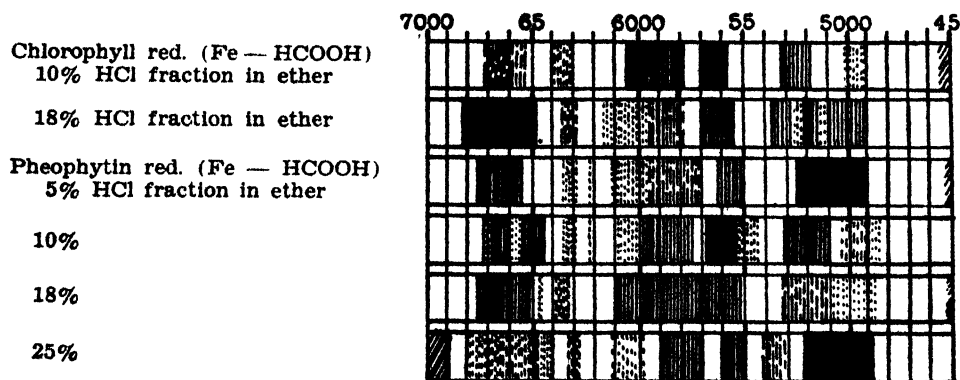


FIGURE 4

Fig. 3 represents the absorption spectra of pure chlorophyll *a* and *b* for comparison with the spectrum of protochlorophyll from corn. The absorption spectrum of the green pigment from the inner coat of hemp seed is also shown. The latter substance was prepared by extracting the seed coats with ether in an extractor. The last two spectra in Fig. 3 represent the absorption of protochlorophyll from pumpkin seed (drawn from Monteverde's data) and of protochlorophyll from corn, both in alcoholic solution.

In order to obtain artificial decomposition products for comparison purposes, the reductions of chlorophyll (*a* + *b*), of pheophytin (*a* + *b*), and of pure chlorophyll *a* were performed.⁴ The properties of the resulting substances differed from the properties observed by Noack. Hydrochloric acid fractionation of the reaction mixtures led to a series of pigments. Fig. 4 records the absorption spectra of some of these fractions.

⁴ Unpublished experiments with the cooperation of J. Hyde and F. Hower.

Fischer (32), upon repeating Noack's experiments, also arrived at results which showed discrepancies. It is difficult to give reasons for these failures to reproduce Noack's findings: there may be a variation due to the amount of iron used (the original reference does not specify a quantity), or there may be a difference in the starting material. The chlorophyll for our experiments was prepared according to the method which Willstätter describes in his book, with the improvements suggested by Schertz (33). The separation of the components *a* and *b* was performed according to Winterstein (34) by selective adsorption from a mixture of the chlorophylls in benzene-petroleum ether solution (1:14 by volume) on dry and finely powdered sugar. None of the pigments prepared by reduction with formic acid and iron underwent color change from red or purple to green when a solution was exposed to light.

An attempt is being made to isolate Timiriazeff's colorless chlorophyll. In these experiments considerable difficulties in handling the reduced chlorophyll were encountered, since its solution is extremely sensitive to oxygen. Our method⁵ is based on Tswett's adsorption analysis.

The absorption spectra of the original and the recovered chlorophyll solutions resemble each other very closely, a fact which has led to the assumption that the two spectra were identical and that the original chlorophyll had been regenerated (Timiriazeff; Kuhn; Albers, Knorr and Rothmund (35)). In their study of the fluorescence spectra of the solutions in different states of the reaction Albers, Knorr and Rothmund have, however, found considerable differences between the original chlorophyll and the recovered chlorophyll. Now the difference has also been established for the absorption spectra. The solution of the reoxidized chlorophyll *a* in ether exhibits positive phase test. The cleavage test yields a relatively small amount of chlorin *e*, the normal cleavage product of chlorophyll *a*, and a larger amount of a substance of chlorin character which can be extracted with 12% hydrochloric acid. The basicity test indicates that the phytol group is still intact.

Fig. 5 shows the absorption spectra of the different products from Timiriazeff's reaction, together with the spectrum of chlorophyll *a* for the purpose of comparison.

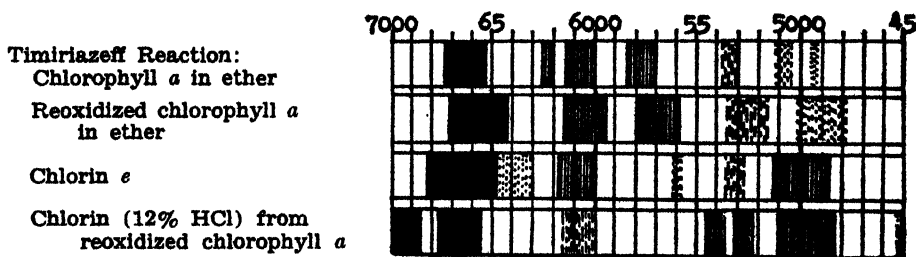


FIGURE 5

⁵ Unpublished; developed with F. Hower and T. Londergan. Reduction, chromatographic separation, and elution of the pigments are performed in the chromatogram tube, in an atmosphere of an inert gas. The tube is filled with the adsorbens (powdered sugar or aluminum oxide is suitable) and a layer of two to three cm. thickness of zinc is placed on top of it. The pyridine solution of chlorophyll, containing the organic acid (in most cases acetic acid) is run slowly through the two layers by suction under nitrogen atmosphere. Reduction takes place while the solution passes the zinc, and the reaction product is immediately adsorbed on the adsorbens in form of a brown zone. Elution under nitrogen with pyridine, acetone, or ether yields colorless or yellow solutions, in higher concentration the liquids are of brown or auburn color. When the tube containing the brown adsorbate is exposed to air, the reduction product turns green in a very short time. The same color change takes place upon exposing the solutions of the reduced chlorophyll to air or oxygen. Attempts to prepare the brown compound in solid form have so far been unsuccessful.

The reduction of chlorophyll with zinc and acid in pyridine solution was also performed in an atmosphere of carbon dioxide. This observation is contrary to Kostytshew's statement that protophyllin turns green in oxygen or carbon dioxide.

In the fluorescence study referred to above, chlorophyll *a* and chlorophyll *b* were subjected to Timiriazeff's reaction in nitrogen and in carbon dioxide, and then reoxidized in air. The fluorescence spectra of the original, the reduced, and the reoxidized solutions were photographed with the set-up described by Albers (36). The densitometer curves on Fig. 6 and Fig. 7 indicate that the reaction in nitrogen atmosphere is different from the reaction under carbon dioxide. This statement holds for chlorophyll *a* as well as for chlorophyll *b*.

For the sake of clearness, each reaction is represented by two diagrams; in one of them, the curve for the recovered material is plotted against

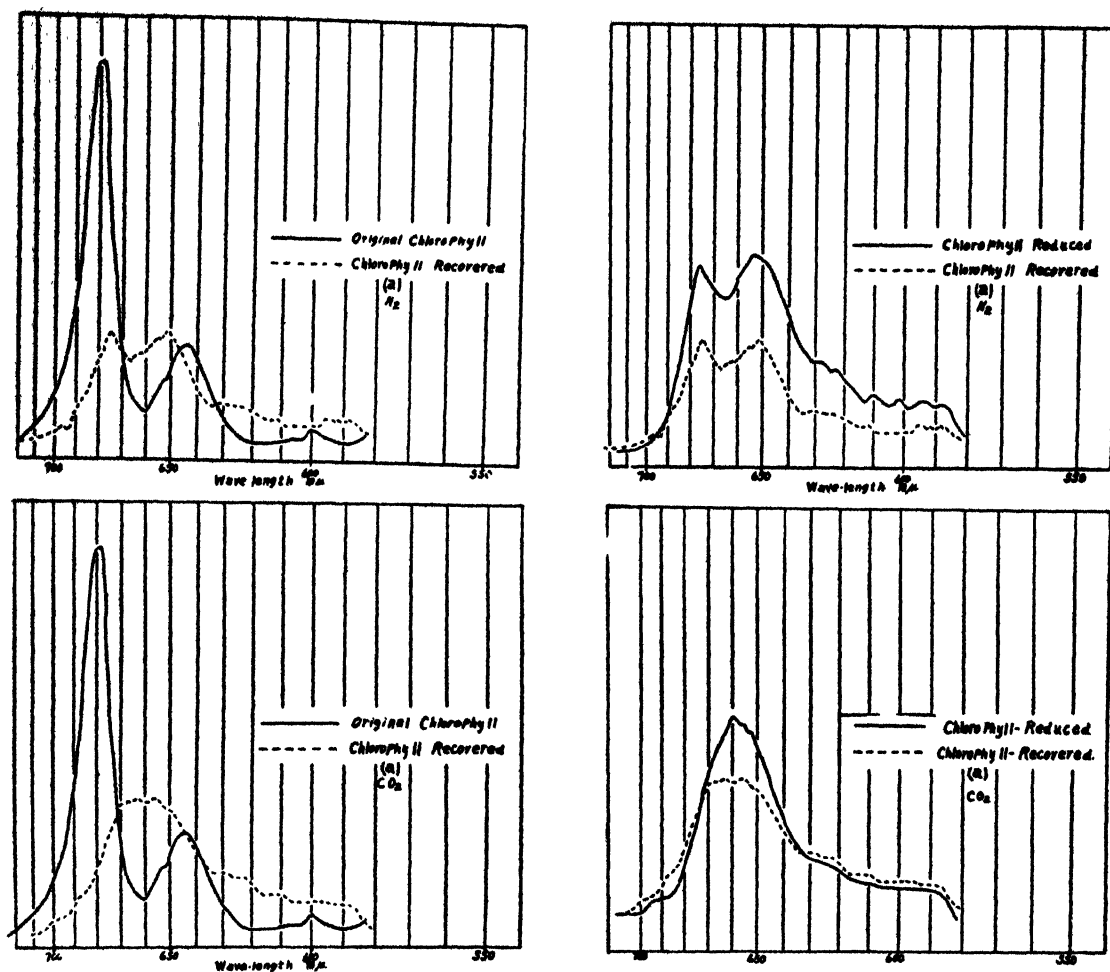


FIGURE 6

the curve for the original substance, in the other diagram, the curves for the recovered and for the reduced compounds are shown. In all cases the maxima of the fluorescence bands of the reduced and of the recovered materials appear at shorter wavelengths than those of the original chlorophyll.

These reduction experiments in nitrogen and in carbon dioxide atmospheres point to the existence of compounds between chlorophyll and carbon dioxide, and between the reaction products and carbon dioxide. In a recent investigation Knorr (37) studied the fluorescence of chlorophyll and some of its derivatives in atmospheres of different gases. His conclusions from the experiments under carbon dioxide support this assumption.

The present status of the protochlorophyll problem may be summarized briefly in the following statements:

1. Protochlorophyll, supposedly the precursor of chlorophyll in the plants, has not been prepared in pure state so far.

2. The reaction:

protochlorophyll from plants \rightarrow chlorophyll
has not been performed *in vitro*.

3. Timiriazeff's reaction cannot be explained as a reversible hydrogenation and dehydrogenation of the chlorophylls. Absorption spectra, fluorescence spectra, and cleavage products indicate that the green pigments in the reoxidized solutions are different from the chlorophylls subjected to the reaction.

4. No chemical reaction is known which would lead from chlorophyll back to protochlorophyll.

5. The chemical character of the green pigment, isolated from etiolated corn by chromatographic analysis, and its physiological role, remain to be determined.

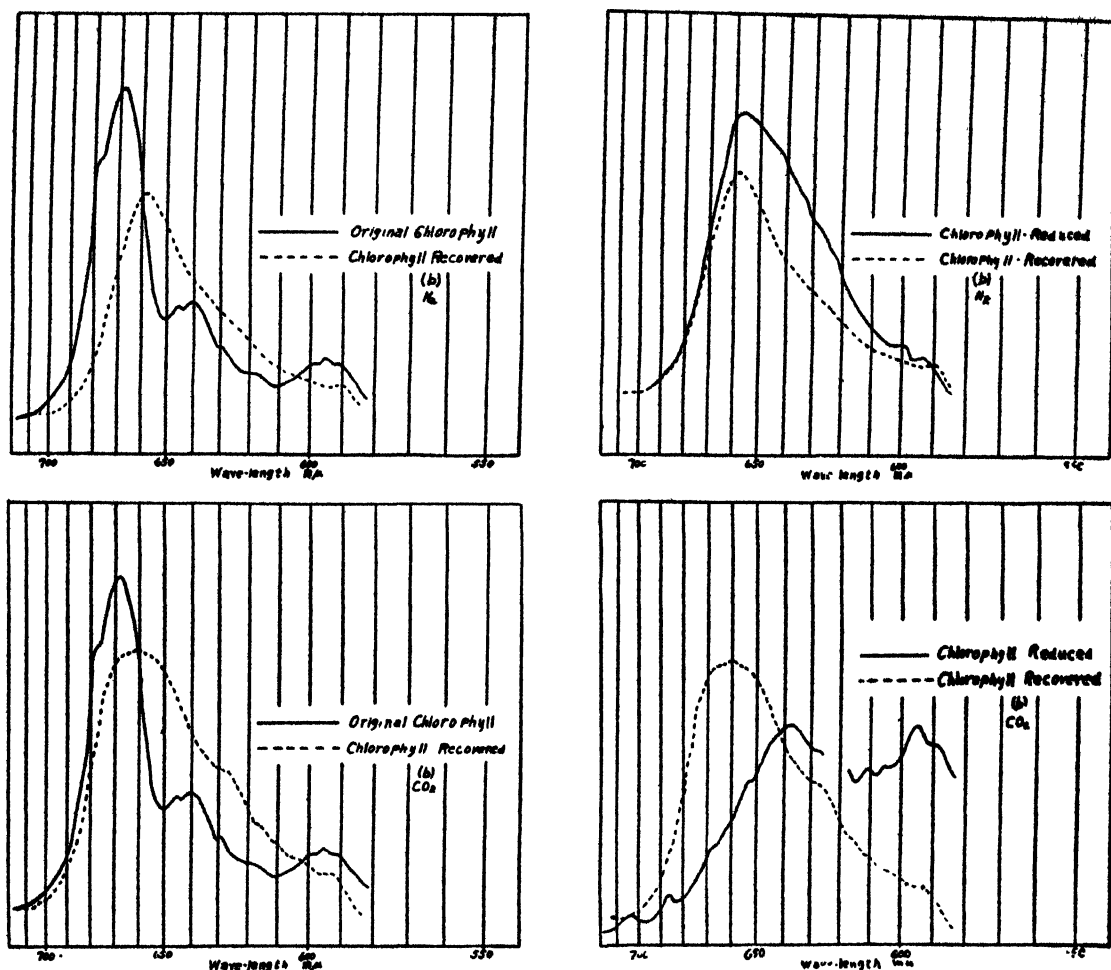


FIGURE 7

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DISCUSSION

Dr. Emerson: Has it ever been shown that the living green plant can change protochlorophyll into chlorophyll?

Dr. Rothemund: No; it has only been demonstrated that after irradiation of the plant the protochlorophyll absorption spectrum becomes weaker and the chlorophyll spectrum appears.

Dr. Emerson: The protochlorophyll does not disappear entirely. Do you usually find it accompanying chlorophyll in living leaves?

Dr. Rothemund: It is probably present in all green leaves along with chlorophyll.

Dr. French: Does the protochlorophyll reform again in the dark, perhaps from chlorophyll?

Dr. Rothemund: It forms again in the dark, whether from chlorophyll, from the precursor, or from both is not known.

⁶I am greatly indebted to Dr. Schertz for his kindness in demonstrating to me his method of preparing chlorophyll in the Laboratories of the U. S. Department of Agriculture in Washington, D. C.

Dr. Burk: Have you any idea of the relative concentration of the protochlorophyll in the plant before it is "changed into chlorophyll"—does it ever attain 75, 10, or 1% of the chlorophyll eventually formed?

Dr. Rothemund: As far as I know, quantitative investigations in this direction have not been published as yet.

Dr. Burk: It is important that the protochlorophyll should have a relatively high concentration in relation to chlorophyll otherwise a precursor of protochlorophyll, existing in greater concentration, might be of considerably greater importance and interest, even though it were more difficult to isolate.

Dr. Emerson: Do technical difficulties or lack of material stand in the way of preparing protochlorophyll in pure form?

Dr. Rothemund: Primarily the lack of material; very large quantities of etiolated corn are necessary to prepare fairly concentrated protochlorophyll solutions.

Dr. Rollefson: It is not surprising to me that the product obtained by reducing chlorophyll with zinc is not reoxidized to the original substance. Zinc is a powerful reducing agent and might be expected to produce several changes in the molecule, some of which will not be reversed by the action of oxygen. With a weaker reducing agent it may be possible to bring about only one change and then there would be a better chance of obtaining the original chlorophyll by reoxidation. The choice of such a reducing agent is rendered difficult by our lack of knowledge concerning electrode potentials in non-aqueous solutions, but qualitative tests should establish the order sufficiently to aid in making this choice. Some of the best prospects would be the more noble metals.

Dr. Mestre: I should think that the distinct differences in the spectra of the original chlorophyll and of the reoxidized material indicate the correctness of Rollefson's contention that the process of reduction used has produced changes in the molecule and that it has not been possible to reverse these by reoxidation.

Dr. Rothemund: Experiments are in progress now to test different reducing agents, particularly mercury.

BEHAVIOR OF CHLOROPHYLL IN INHERITANCE

M. DEMEREC

In this review there is no intention either to go into the details of genetic analysis of various chlorophyll types or to present a complete review of the genetic work done on chlorophyll. The primary purpose is to point out to students of physiology and chemistry of chlorophyll the existence of internal, hereditary factors responsible for profound differences in chlorophyll content in various plants, and to stress the fact that an abundance of genetically uniform material may easily be obtained for chlorophyll studies.

Genetics of chlorophyll has been most intensively studied in maize. Fortunately this plant is also convenient for other experimental studies, because, due to its fast growth and easy culture, an abundant supply of material can readily be obtained. For these reasons, I shall center my discussion on maize and shall refer only occasionally to work done on other plants.

A general review of chlorophyll inheritance has been prepared recently by de Haan (1933), and a list of chlorophyll characters of maize with short descriptions has been published by Eyster (1934).

Heredity of chlorophyll types. In inheritance, chlorophyll shows more peculiarities than any other known plant characteristic. In addition to a large number of Mendelian types, there is known among chlorophyll characters a large group of types which are inherited in a non-Mendelian fashion. In that group the bearers of heredity appear to be extranuclear and, in certain cases, it seems probable that plastids may be responsible for the transmission of the character. These non-Mendelian types are of interest for students of heredity, but they shall not be reviewed here, since they could play, at best, only a secondary role as material for chlorophyll investigations. I shall therefore limit this presentation to Mendelian chlorophyll types.

In inheritance, chlorophyll behaves like any other characteristic such as anthocyan color, leaf shape, size, flower shape, etc. Its presence or absence, and also its concentration, are influenced by numerous genetic factors (genes) which are inherited in the usual manner. The presence of chlorophyll in the majority of plants and its importance in the vital processes of green plants do not in any way decrease the role which various genes play in its expression. Just the opposite seems to be true. The wide distribution of chlorophyll and the essential part it plays in the life of green plants are undoubtedly responsible for the existence of a large number of genes having a striking effect upon it.

In the case of chlorophyll, as in other characteristics, the most common types are simple Mendelian recessives, indicating that the physiological disturbance causing the appearance of a particular characteristic is usually the result of the action of one gene. A number of cases are known, however, where two or three genes are responsible for the appearance of the characteristic. Since most inherited characters affecting chlorophyll are detrimental to the plant, a strong selection against dominant types is effective under ordinary conditions. Such types, therefore, one would expect to be rare. They have been described in *Antirrhinum* (Baur 1907), *Urtica* (Correns 1922), *Crepis* (Collins 1924), and *Nicotiana* (Lodewijks 1911). In maize, a dominant-lethal pale green character has been observed on a plant in which it originated (Kempton 1924).

It is commonly found among chlorophyll characters that types identical in appearance are determined by different genes. For example, in maize, 13 genes are known, each of which independently produces albinos (Demerec 1926), 20 are known to produce virescent types (Phipps 1929), 30 various pale greens (Maize circular letters), 4 piebald seedlings (Demerec 1926a); and 4 zebra stripings (Demerec 1924). A similar condition was discovered in sorghum (Karper and Conner 1931), and could undoubtedly be found in other plants if an intensive study were made.

Description of chlorophyll types. It is usually true that in any group where a large number of types is available, classification into sharply limited classes is not possible because of the existence of intermediate forms. In such cases, the division line between classes is of necessity arbitrary. Such a situation is found in chlorophyll. Known chlorophyll types can be grouped into several apparently distinct groups, but intermediate forms are known to exist.

For genetic purposes chlorophyll types are divided according to their appearance at certain stages of development of the plant. In a few instances an attempt was made to make qualitative and quantitative measurements of the plastid pigment. In spite of the fact that the technique used in these measurements was so crude that the results only approximate the actual conditions, the analysis gave a means of separating chlorophyll types on a more exact basis than mere appearance. This method has been particularly used in the case of pale green types. It consists of extracting the pigment with ethyl alcohol,

separating green and yellow parts with petrol-ether, and colorimetrically comparing the extracts of normal and pale green seedlings.

For the purpose of this review chlorophyll types will be divided into four groups viz:

1. albinos, in which plastid pigment is absent and which because of the lack of chlorophyll, die in an early seedling stage;
2. virescent types, which are albinos in the early seedling stage but develop pigment later;
3. pale-green types, in which the quantity of pigment is reduced at certain stages of the development; and
4. variegated types, in which the chlorophyll content differs in different regions.

I shall briefly describe here the principal characteristics peculiar to the types belonging to each of these groups.

Albinos. Albinos are the most frequently found of abnormal chlorophyll types. They have been observed in so many species that the conclusion is justified that they may occur wherever chlorophyll is present. In maize, albinos are present in a heterozygous state in many commercial varieties (Hutchison 1922) from which they can be brought out by inbreeding. Experiments indicate that a large proportion of these albinos is determined by genetically different factors (Demerec 1923, 1926). In maize at least 13 such factors are known at the present time (Demerec 1926), and only the amount of labor required to complete the necessary tests is keeping this number from being greatly increased.

In the early seedling stage, albino seedlings are snow white since they possess practically no plastid pigment. The seed, producing albinos, germinates at the same time as the seed producing green seedlings, and in certain lines, at least, albino seedlings grow at the same rate as green seedlings until they reach the three leaf stage when they stop growing and die. The reserve food present in the seed is apparently sufficient to keep the seedlings growing until the three leaf stage. Since albino and green seedlings do not differ in rate of growth during their early life, it seems probable that the assimilation products of young seedlings do not contribute significantly to their growth. In certain albinos, chlorophyll begins to appear at the tips of leaves just before the death of the seedlings. It does not develop sufficiently, however, to supply the necessary food material after the reserve food from the seeds is exhausted.

A cytological investigation of albino seedlings of maize was made by Miles (1915) and later also by Randolph (1922). Miles concluded that plastids are entirely absent from albino plants.

Randolph's studies revealed, however, that the plastid primordia proceed to develop at the same rate in albinos as in green seedlings, but in the case of albinos this development stops and a degeneration process begins approximately at the stage comparable to that at which chlorophyll appears in a green plant. Randolph's conclusion is that the failure of the plant to become green is not due to the absence of plastids or plastid primordia.

Albino seedlings, therefore, possess in a rudimentary state the organs (plastids) in which the color develops, but lack some physiological mechanism essential for the development of plastid color. This condition is brought about by the genetic constitution of the plant.

Several genes are known in maize which, when present together with the gene for albinism, induce the development of yellow pigment in albino seedlings. Such seedlings have a lemon yellow color and are called luteus seedlings. They die because of the lack of chlorophyll. According to Lindstrom (1918), yellow pigment of this type closely resembles xanthophyll. It is insoluble in water and weak alcohol, but readily dissolves in strong solutions of ethyl alcohol (95%).

An albino line is propagated through the green sister plants of albinos. A selfed green plant heterozygous for an albino gene will transmit albinism to one quarter of its offspring; two thirds of its green offspring will again be heterozygous for albinism and will thus be able to propagate the albino line.

Virescent seedlings. The main characteristic of virescent seedlings is a delay in chlorophyll development. In early life certain types look like albinos, but as they grow older, chlorophyll begins to develop in the tips of the leaves and soon the whole seedling becomes green. New leaves, appearing after a seedling becomes green, are also green, and the mature virescent plants do not differ in green color from normal green plants.

Since virescents of maize are among the most useful characters for genetic research, they have been intensively studied, and so far twenty genetically different virescent types have been established (Phipps 1929). Many of these types differ greatly from each other in the rate at which the chlorophyll develops. At the one extreme, are plants which become green only if grown under favorable conditions, and at the other extreme plants which under the same circumstances do not show the albino characteristics at all. Virescent types are known in which chlorophyll development does not proceed sufficiently fast for seedlings to live. Such types, however, are less desirable from the genetic standpoint, and they have not been included in the material studied.

It has been observed that virescent seedlings become green sooner if grown in the summer than if grown in the winter. Experiments indicate (Demerec 1924a) that temperature is responsible for this difference. The other factors tested were light duration and light intensity. It has been noticed that different virescent types show differences in their reaction toward temperature.

Pale-green types. To a physiologist or a biochemist this group offers interesting material because of the variety of types differing in the amount and composition of plastid pigments. In maize about 30 genetically different pale green types are known. Certain of them do not live beyond the seedling stage, others are pale green as seedlings but turn green later, still others are pale green both as seedlings and as mature plants, and the last group have green young plants which become pale green as they reach maturity. For a number of these types an estimate of chlorophyll content was made. Since this knowledge is of particular interest to students of chlorophyll these types will be described here in detail.

The results of chlorophyll analysis are shown in Table 1.

served by Correns (1919) in the case of the xantha character of *Capsella*. Xantha plants grow better if they are not exposed to full sunlight.

Pale green-1, 3, 4 and 5 types do not live beyond the seedling stage. Chlorophyll content analysis indicates that they have more pigment than the xantha types, but they nevertheless all die as young seedlings. Especially interesting in this respect is pale-green 5. Chlorophyll analysis indicates that the seedlings of this type have about 71-83% of normal chlorophyll content. In the early seedling stage they look so much like green seedlings that they can be distinguished from them only with difficulty, but as they grow older they begin to appear water-soaked and invariably die before reaching the age of four or five weeks. Seedlings were grown under various environmental conditions, but no conditions were found favorable enough for survival.

It is of interest to note that pale-green-5 seedlings, which possess about 71-83% of chlorophyll, die at about the same stage as do albinos, which do not possess any chlorophyll at all. Both of them die in the three leaf stage apparently

TABLE 1. *Chlorophyll content of different pale green types of maize.*

Name	Symbol	Percentage of Pigment		Authority	
		Green	Yellow		
Xantha-1	xn-1	10.7	normal	Trajkovich	1924
Xantha-2	xn-2	10	normal	Demerec	1925
Pale green-1	pg-1	15	50	Brunson	1924
Pale green-2	pg-2	53	normal	Demerec	1925
Pale green-3	pg-3	28.2-30.7	normal	"	"
Pale green-4	pg-4	38.5-50.5	normal	"	"
Pale green-5	pg-5	71.5-83.5	normal	"	"
Golden-1	g-1				
upper leaves		50	normal	"	"
lower leaves		8.7	normal	"	"

The two numbers in the percentage column stand for the highest and lowest values in cases where determinations were made several times by using different material. All of these plastid pigment determinations were made by the method mentioned earlier.

Both the xantha-1 and the xantha-2 types are pale green throughout the life of the plant. Xantha plants reach maturity but they are very weak because of the deficiency in chlorophyll content. It is interesting to note that about 10% of the normal chlorophyll content is sufficient to keep a plant alive. A similar condition has been ob-

served after the reserve food from the seed is exhausted. A close examination of the dying pale-green plants shows that they have a well developed root system, but that the roots are practically dead, just as in the case of the albinos. This may indicate that the chlorophyll in the pale green-5 does not develop to the stage at which it is capable of photosynthesis and that the roots and leaves die due to lack of food. It is highly improbable that the lack of chlorophyll is the cause of the death of these seedlings, since xantha types possessing only about 10% of normal chlorophyll content are able to live to maturity.

The golden type referred to in Table 1 was first described by Lindstrom (1918). Usually golden plants are normally green in the seedling stage, although some of them, when grown under special conditions, appear yellowish. After a plant is a month or more old, the green color begins to disappear, gradually giving rise to a yellow-green and finally to a yellowish golden color. The first indications of the change appear in the tips of the older leaves, then gradually the chlorophyll decomposes or disintegrates, the yellow color extends to all the leaves, and at the same time the stalk becomes yellow. Plastid pigment determination (Table 1) was made when golden plants were in the tasseling stage, when the upper leaves had just begun to lose their chlorophyll and the lower ones were already golden yellow. At that time the upper leaves had about 50% of the green pigment and the lower leaves about 8.7% while the amount of the yellow pigment appeared to be normal.

Variegated types. A number of variegated types with normal and chlorophyll deficient tissues adjacent to each other are known in maize and also in many other plants. In the case of maize variegated types vary in regard to pattern, from those with fine streaks to those with wide bands of contrasting tissue, and in regard to the plastid pigment, stripes may be either albino, yellow, or pale green of different intensities.

To those working with plastid pigments the japonica type of maize may be useful for certain experiments. Japonica type is well known and is frequently used for ornamental purposes. The plants of this type have longitudinal stripes which may be white or yellow. If a genetic factor for the anthocyan pigment is present in such plants, white stripes become red giving the plants an effective appearance for ornamental purposes. The stripes vary in width from narrow streaks to wide bands covering one half of a leaf or even more. Very few seedlings show striping, since stripes develop mostly on late appearing leaves. When the luteus factor is present in japonica plants, stripes which are usually white become yellow, because the luteus factor permits the development of the yellow plastid pigment.

Another group of variegated types of maize which may be of interest to the students of plastid pigment are piebalds. Three of them have been described (Demerec 1926). Leaves of these types have large blotches devoid of plastid pigment. Piebald-4 is particularly prominent and shows blotches both on seedlings and on the mature plants.

Discussion. It is an established fact that chlorophyll may be visibly affected by a large number of genes. Some of these genes prevent the de-

velopment of chlorophyll entirely and thus are responsible for the appearance of albinos, others delay the chlorophyll development and produce virescent types, others partially reduce the chlorophyll content causing the appearance of pale-green types, and finally, some of them produce regional effect and are responsible for various variegations. This shows that the plastid pigment can undergo a variety of transformations through changes in the hereditary constitution of the plant. The available evidence is definite to show the existence of quantitative differences between chlorophyll types, and certain results may be interpreted as due to the existence of qualitative differences in the plastid pigment. To establish such differences, however, a special technique is required which is not adjusted to the facilities of geneticists studying this problem. Biochemists interested in the chemistry of chlorophyll may find the genetically uniform material accumulated by geneticists a gold mine for their investigations, just as the study of chlorophyll inheritance is a gold mine for geneticists.

Genetic research made it evident that no single gene is responsible for the appearance of any one characteristic. The phenotype of an organism is determined by the interaction of the whole complement of genes which the organism possesses. These genes form a balanced system, and any changes in the genes disturb this balance; this disturbance may be registered by the organism as a definite characteristic. The appearance of a chlorophyll character, therefore, may be visualized as caused by a disturbance in the physiological balance within the organism due to a change in a certain gene. Since a gene probably has a specific action, a change in a certain gene is likely to affect a certain type of reaction and thus to exert a major influence on a single characteristic.

It is known that several genetically different changes may produce a very similar visible effect on the organism. For example, 13 different genes are known in maize, any one of which when present is responsible for the appearance of albinism. This indicates either that these different genes are responsible for similar reactions resulting in albinism or that a similar end-product may be produced in several different ways. Both possibilities are probable and undoubtedly both of them do occur. In the case of albinism it may be possible that the character is brought about through some physiological condition which prevents the development of the plastid pigment, or it is possible that a certain physiological condition is detrimental to the growth of plastids and therefore the pigment normally present in them is not able to develop. Again, it may be possible that a certain gene causes the absence of plastids. All

such and similar conditions would produce the same end-effect, although the processes responsible for it would not be related. A similar situation is possible in the case of other chlorophyll types. All this points to the advisability of following in chlorophyll experiments a rule which was found to be very important in many other studies viz: that genetically known and uniform material should be used for experimental purposes. To those conducting breeding work with maize this belief is strengthened through the everyday experience of observing fairly striking differences in green color of various families. As previously pointed out, qualitative differences in the pigment may be responsible for these color differences, in which case results of chemical analysis may be affected by the type of material used.

A comparison of the size of plastids of various chlorophyll types of maize indicates that the size of plastids is correlated with the amount of pigment present. Trajkovich (1924) determined the chlorophyll content and measured the size of plastids of four chlorophyll types of maize and found a correlation between the two. Trajkovich's data are given in Table 2.

TABLE 2.

Chlorophyll analyses and plastid measurements of different chlorophyll types of maize.

Type	Relative Amount of Pigment Present		Average Size of Plastids (Microns)
	Green	Yellow	
Green	100	100	6.19 ± 0.42
Xantha	19.7	100	4.62 ± 0.04
Yellow	6.1	100	3.19 ± 0.04
White	0	0	Primordia

From this discussion it is evident that the presence or absence of chlorophyll in plants is determined by the selective value of the character. Every plant, apparently, possesses the mechanism for developing a race devoid of chlorophyll. Since the life of the great majority of plants is not adjusted to the chlorophyll-less condition, races without chlorophyll are not found. If, however, albino plants were able to survive, albinism in chlorophyll would be more frequent than the albinism among flower colors.

I will conclude this presentation by summarizing briefly the results of the genetic analysis of chlorophyll in maize. About one hundred genetically different chlorophyll characters are known in that plant, some of which manifest themselves in the seedling stage only, others in the mature

plants only, and still others appearing both in seedlings and in mature plants. In regard to the chlorophyll content they range from albinos, which are entirely devoid of chlorophyll, through virescent types in which the development of chlorophyll is delayed, to pale greens, which again vary from types with very little pigment to those having almost the normal amount. Also a large number of variegated types are known with regions in which the pigment is reduced. In each of these groups several characters are known similar in appearance but differing in their genetic constitution.

All this material offers an almost virgin field for biochemical and physiological studies of chlorophyll. It seems to me that a cooperative effort of geneticists, physiologists and biochemists would be highly profitable in utilizing to the limit the great opportunities available in this field.*

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* **Demonstrations.**—When this paper was read, the following chlorophyll types were shown as growing seedlings. The seed for the majority of them was obtained from Dr. M. M. Rhoades, formerly of Cornell University and now of the U. S. Department of Agriculture, Ames, Iowa.

1. Albino seedlings (w11), 2. Luteus seedlings (1,1₁ w₁w₁), 3. Virescent-2 (v2), 4. Virescent-4 (v4), 5. Xantha-2 (xn 2), 6. Pale-green-3 (pg 3), 7. Pale-green-4 (pg 4), 8. Virescent-pale-green, 9. Piebald 4 (pb 4), and 10. Yellow striped-1 (ys 1).

DISCUSSION

Dr. Harris: All the chlorophyll differences in Table I seem to be quantitative. You mention that certain results may be interpreted as due to the existence of qualitative differences in plastid pigments. Would you describe these results?

Dr. Demerec: Xantha types with about 10% of chlorophyll are able to live to maturity, while some pale green types with as high as 80% of chlorophyll die in the early seedling stage. If this death is due to chlorophyll, that would suggest that chlorophyll in xanthas and lethal pale-greens is qualitatively different.

Dr. Harris: Have any spectroscopic studies been made?

Dr. Demerec: Not so far as I know.

Dr. Emerson: Have any of the albinos been kept alive by feeding with organic material?

Dr. Demerec: It has been tried, but without success.

Dr. Emerson: Have the cultures been free of bacteria?

Dr. Demerec: I doubt if cultures free of bacterial have been used.

Dr. Emerson: From these experiments on maize, it seems to me one cannot be sure that lack of the products of photosynthesis causes the defective seedlings to die in early stages. Do you think inability of the albinos to take necessary elements in through roots might be the cause of their premature death?

Dr. Demerec: Yes, very likely, in some cases at least. There are, however, many hereditarily unrelated albinos, so that it might be expected that in all cases the inability to absorb nutrients would not go together with the deficiency in chlorophyll.

Dr. Inman: Corn is very inefficient in absorbing organic nutriment through its roots.

Dr. Emerson: Have you tried floating the leaves on sugar solutions?

Dr. Demerec: I do not recall whether that has been tried or not.

Dr. Marshak: In the case of the virescents, the first appearance of chlorophyll in the tips of the leaves suggests that an inhibitor of chlorophyll may be transported from the region of the endosperm into the leaves. If that were the case, one might expect that with the endosperm removed the chlorophyll would develop sooner. Has that been tried?

Dr. Demerec: I think it has not been tried.

Dr. Harris: The variegated forms indicate that cells, lacking chlorophyll, whether albinos or luteus containing cells, can live, provided they receive sufficient nutriment. How sufficient nutriment can be supplied experimentally in the case

of plants which are completely albinos or luteus, is a question.

Dr. Demerec: The only successful way known to me to keep albinos alive is through grafting. Since in monocotyledons grafting is not possible, this method cannot be used in maize. It has been used, however, by Dr. Blakeslee in *Datura* and also by others in certain other plants.

Dr. Marshak: Has anybody studied respiration rates of these different types of seedlings?

Dr. Demerec: Not with maize.

Dr. Inman: You spoke of multiple genes in some instances. Do they have cumulative effects?

Dr. Demerec: No. There are a few cases known where the chlorophyll character is determined by duplicate genes in which both genes have to be present in order that the character appear.

Dr. Brackett: Are there any cases where the amount of carotinoid pigments is reduced in relation to the amount of chlorophyll?

Dr. Demerec: No. None are known. From the genetic standpoint there is no *a priori* reason why they should not exist. In any case they would not be easy to detect by mere appearance.

Dr. Brackett: One thing we would like to have is a plant in which the carotinoids are suppressed and the chlorophyll normal. One wonders whether the carotinoids are involved in the synthesis of chlorophyll so that this condition of relatively low carotinoid may never be possible.

Dr. French: Is the existence of albino and luteus forms evidence that genes for production of chlorophyll and production of carotinoids are separate?

Dr. Demerec: It shows that we know genes which affect the development of the yellow pigment without affecting the development of the green pigment (luteus genes). If such a gene is present in an albino plant, yellow pigment will develop.

Dr. Marshak: If you were to extract the normal sibs of the white albinos and the yellow and found in both cases the same amount of carotinoid pigments, it seems to me that you might obtain some information about the development of chlorophyll. The question has been raised as to whether the carotinoids may be precursors of chlorophyll or are unrelated products. If the extracts of the normal luteus sibs showed a higher carotinoid content in proportion to chlorophyll than the normal sibs of the albinos it would indicate that the carotinoids were probably not precursors of chlorophyll. The problem might also be approached by studying the rate at which chlorophyll was developed in these normals after the seedlings had been grown in the dark. One might expect that chlorophyll would be developed more rapidly in the luteus sibs.

Dr. Harris: Perhaps it should be mentioned that if these interesting experiments were conducted, and even if a higher carotinoid content in proportion to chlorophyll were found in the luteus sibs, one would not necessarily obtain evidence that carotinoids were not precursors of chlorophyll, particularly in the event that the rate of chlorophyll development was not changed. Furthermore, the presence of an "excess" amount of precursor is a normal occurrence, if, as Dr. Rothenmund states, in the discussion of his paper, the precursor, protochlorophyll, is always, (as far as is known) coexistent with chlorophyll in green leaves. Plants somewhat analogous already exist in nature, as Dr. Demerec has found; namely plants having 100 per cent luteus pigment and only 10 per cent of the normal amount of chlorophyll.

Dr. Marshak: Has anybody extracted the normal sibs of the luteus and the white albinos?

Dr. Demerec: Not so far as I know.

Dr. Harris: Is it known whether the thirteen different albinos act physiologically the same way or in different ways?

Dr. Demerec: Physiological activity of albinos has not been investigated. This would be an interesting problem.

Dr. Brackett: It would be very interesting to make photosynthesis measurements on the pale green types in order to compare those which are able, and those which are unable, to grow to maturity, both of which show some chlorophyll pigmentation.

Dr. Inman: Do all inbred strains develop albinos to the same extent? We have several inbred strains and we seldom see an albino.

Dr. Demerec: Albinos, if present in commercial varieties, can be brought out through inbred-

ing. In inbred strains it is very easy to select against albinos and to eliminate them.

Dr. Harris: Is the variegated type due to unstable genes?

Dr. Demerec: Not chlorophyll variegations known in maize. There are several such variegations known in other plants which are due to unstable genes.

Dr. Inman: Would you care to say anything about the question of the inheritance of increased chlorophyll content?

Dr. Demerec: I do not know of any work to increase the chlorophyll content in maize. Corn breeders try to develop higher yielding varieties and I do not know whether higher yield is correlated with high chlorophyll content or not.

Dr. Smith: Are pale green plants due to reduced number of chloroplasts or to a smaller amount of chlorophyll in each chloroplast?

Dr. Demerec: Observations made by Trajkovich suggest that smaller amount of pigment in chloroplasts is responsible for pale green appearance.

Dr. Inman: Have you made any studies of chlorophyll deficiency in teosinte?

Dr. Demerec: No.

Dr. Moyer: Since many of these plants seem to have sufficient chlorophyll and yet are unable to use it to keep alive, have any studies on the structure of the plastid in those deficient forms been made?

Dr. Demerec: No.

Dr. Harris: I would like to express my complete support of Dr. Demerec's suggestion of the value of physiologists' and biochemists' using genetically tested material whenever feasible. The material described by Dr. Demerec would seem to be very suitable for certain chlorophyll studies.

FLUORESCENCE AND PHOTODECOMPOSITION OF THE CHLOROPHYLLS AND SOME OF THEIR DERIVATIVES IN THE PRESENCE OF AIR

V. M. ALBERS AND H. V. KNORR

INTRODUCTION

The red fluorescence of solutions of chlorophyll is a very obvious physical property. It was observed as early as 1833 by Brewster¹ and was first studied by Sir G. G. Stokes² in 1852. Stokes studied the fluorescence by irradiating the solutions with the spectrum of sunlight. He observed a red band of fluorescence with the first absorption band at about its center. He also observed that the modified radiation was always of longer wavelength than the unmodified radiation. He came to the conclusion, from his studies

of the optical properties of the substance then known as chlorophyll, that it consisted of a mixture of substances and he contrived by the use of two immiscible solvents (alcohol and carbon disulfide) to separate this mixture into four separate pigments, two yellow and two green. It is interesting to note that Stokes probably used more nearly pure chlorophyll than anyone up to the time of Tswett³ in 1906 and Willstätter⁴ in 1911. In 1864 Stokes^{5,6} proved that biliverdin, the green bile pigment, was not identical with chlorophyll, as Berzelius supposed, by comparing

TABLE I

Crude Leaf Extract							
Name	Date	Wavelengths in m μ				Remarks	
Hagenbach ^(7,8)	1870 1872	679	650	578	560	526	Ether Soln.
Lommel ⁽⁹⁾	1876	675					Used monochromatic radiation Found Stokes' Law did not hold.
Lubarsch ⁽¹⁰⁾	1879	686	645	614	593	565	501 Excited by λ 665.
Nichols and Merritt ⁽¹¹⁾	1904	718					Chlorophyll was probably allomerized
Pure Chlorophyll <i>a</i>							
Dhéré ⁽¹²⁾	1914	664					Ether Soln. Fluorescence excited by carbon arc. $\lambda < 470$
Knorr ⁽¹³⁾ and Albers	1932	734	679	672	633		Ether Soln. Fluorescence excited by visible radiation from mercury arcs.
				677	636		Benzene Soln.
				672	639		Acetone Soln.
				675			Methanol Soln.
Albers and Knorr ⁽¹⁴⁾	1934						No new wavelengths. Emphasis on Photodecomposition.
Zscheile ⁽¹⁶⁾	1935	723	668.5				Ether Soln. Fluorescence excited by total radiation from incandescent lamp
Pure Chlorophyll <i>b</i>							
Dhéré ⁽¹²⁾	1914	647					Same as above
Knorr and Albers ⁽¹⁵⁾	1934						See Table II
Zscheile ⁽¹⁶⁾	1935	705	672	648.5			Same as above

Table I. Summary of the determinations of the wavelengths of the bands in the fluorescence spectra of chlorophyll *a* and *b*

their absorption spectra and observing that the biliverdin was entirely lacking in the red fluorescence so characteristic of chlorophyll. Stokes' spectroscopic equipment was limited to a glass prism used with the naked eye. He was not able, therefore, to observe very accurately the nature of the fluorescence spectrum, beyond the fact that it consisted of a broad red band.

Since the time of Stokes the fluorescence spectrum has been studied by several observers. Table 1 gives a summary of these observations in chronological order with the wavelengths of the bands observed. The first five observers used a crude extract of plant leaves. The other observers all used chlorophyll free from other impurities and the two components, *a* and *b*, were studied separately. The most striking characteristic of this table is the lack of agreement between the different observers on the wavelengths of the bands observed. The reasons for this disagreement will become evident as the data in this paper are discussed.

The photodecomposition of chlorophyll solutions was probably first observed by Senebier¹⁷ in 1788. He observed that solutions of chlorophyll in acetone, alcohol, benzene and ether were rapidly decolorized by light in the presence of air.

Studies of the photodecomposition of chlorophyll have been made in which the process has been carried out under other atmospheres than air. These studies will be reviewed by Dr. Knorr in the following paper. The general conclusions from these studies, however, is that the photodecomposition is an oxidation process.

Wurmser¹⁸ has studied the photodecomposition as a function of the wavelength of the light used. He found that the amount of decomposition which takes place depends only on the amount of energy absorbed and is independent of the wavelength. He also concluded that the decomposition is an oxidation process. It is well known that the chlorophyll is not nearly so susceptible to photodecomposition in the living leaf as it is in solution in organic solvents. If water is added to an acetone solution of chlorophyll, a colloidal solution is formed and in this form the stability of the chlorophyll in the presence of light is much increased. Wurmser tried the addition of colloids such as casein, gelatin, albumin and gum arabic. He found that very small percentages of these substances made a solution of colloidal chlorophyll very much more stable in the presence of light. He found, however, that the addition of starch had practically no effect on the stability of the chlorophyll. As a result of these experiments Wurmser suggested that the chlorophyll in the living leaf is probably protected

against photodecomposition by the presence of colloids.

EXPERIMENTAL

A diagram of the apparatus used is shown in Fig. 1. Four, 150 watt, pyrex mercury arcs

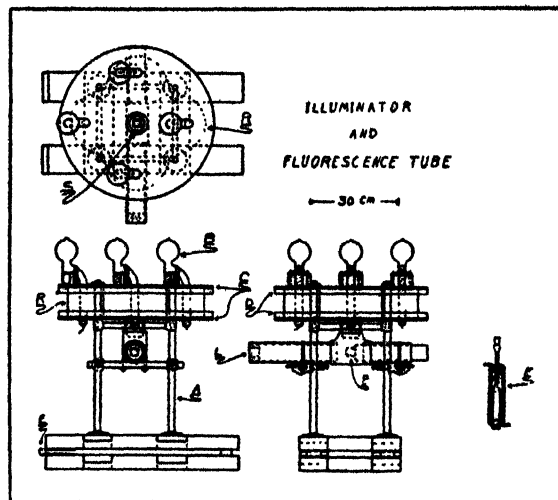


FIGURE 1.

were used, and the system of reflectors was similar to that used by Anand^{19,20}. The fluorescence spectra were photographed with a Hilger E₈ spectrograph using Ilford hypersensitive panchromatic plates. The exposure times varied from five to fifteen minutes according to the intensity of the fluorescence of the substance being studied. All of the substances studied were made up into solutions with a concentration of one milligram per 100 cc. of the solvent. Photographs of the fluorescence spectra were made at intervals during the photodecomposition, the length of the interval depending on the rate at which the photodecomposition took place. The fluorescence spectra were analyzed with a point by point densitometer and the curves showing darkening on the plate as a function of distance along the plate were plotted on large sheets of graph paper.

The substances which have been studied are chlorophyll *a* and *b*, methyl chlorophyllide *a* and *b*, pheophytin *a* and *b*, methyl pheophorbide *a* and *b*, and pheophorbide *a* and *b*. These substances may be divided into two groups according to whether Mg is present or absent. The Mg-containing substances are called chlorophyllides and those not containing Mg are called pheophorbides. The formula for chlorophyll *a* (phytyl chlorophyllide *a*), according to Fischer²¹, is given in Fig. 2. Methyl chlorophyllide *a* is formed by replacing the phytyl group with a methyl group. Pheophytin *a* (phytyl pheophorbide *a*) may be

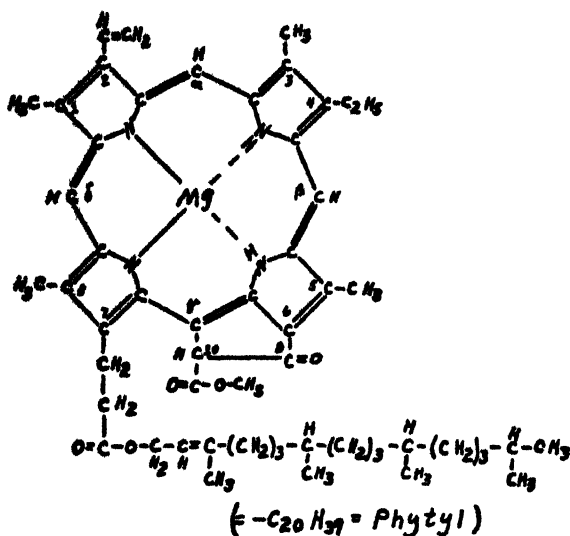
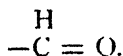
Chlorophyll *a*

FIGURE 2.

formed from chlorophyll *a* by replacing the Mg with two H atoms. In methyl pheophorbide *a* the phytol is replaced by a methyl group and in pheophorbide *a* it is replaced by an H. The corresponding *b* series is formed by replacing the methyl group at position 3 (see figure) by the formyl group



In order to study the role played by the solvent in the fluorescence and photodecomposition, four different solvents, ether, benzene, acetone and methanol, were used with chlorophyll *a* and *b*. The chlorophyll in the form of the normal *a* and *b* mixture was kindly furnished by Dr. Frank M. Schertz of the U. S. Department of Agriculture. This mixture was separated by the methanol procedure of Willstätter as described in his book²². The other substances were all prepared and separated into the *a* and *b* components according to the procedures given in Willstätter's book*.

RESULTS

It is obviously impossible to reproduce all of the densitometer curves which were obtained. In

* In all cases the substances used showed a positive Molisch reaction (phase test) and they showed the correct absorption spectra. In the case of chlorophyll *a* and *b* the cleavage test and the basicity test were made in addition to the other tests. All of the work of preparation and separation of the substances as well as the testing was done either by or under the direction of Dr. Paul Rothemund.

order to show how the fluorescence varied during the photodecomposition a representative set of these curves was selected for each run and reproduced on charts with wavelength scales drawn in. The curves selected to make up a chart were not always selected at equal time intervals because sometimes there are periods during which the fluorescence changes more rapidly than others. Figs. 3-6 show the charts of the densitometer curves for the substances which have been studied. Darkening on the plate is plotted as ordinates and distances measured along the plate are plotted as abscissae. A wavelength scale is superimposed on each of the curves. Each curve has a time interval marked in hours and minutes which indicates the time elapsed since the beginning of the photodecomposition as well as the time of exposure of the spectrogram. On examining the charts in Fig. 3 we find that the first fluorescence spectrogram for each solvent shows two principal maxima except in the case of methanol. Even in this case there is a slight indication of a second maximum on the first curve. As the photodecomposition proceeds the shape of the densitometer curves changes and, in general, the resolution of the two bands becomes less pronounced while the wavelength of the maximum of the band farthest toward the red shifts toward the violet. The curves for the benzene solution show quite definitely the appearance of another band between the two original bands at the time 1' 00" and 2' 00". This third band apparently builds up as the photodecomposition proceeds and it quite easily accounts for the shift in position of the maximum of the first red band. The same process can be seen, although not so clearly, in the case of ether and acetone solutions. Because the methanol solution hardly shows resolution of the two original bands it is impossible to see the third band on the curves. In order to show the changes in wavelength of the bands during photodecomposition for the solutions of chlorophyll *a* in the four different solvents the chart, Fig. 7, has been prepared. In this chart the readings of the positions of the two principal band maxima for the spectrograms made during the first eight hours of photodecomposition are shown. The first spectrogram for each solvent was made during the time interval zero to five minutes, the second in the interval fifteen to twenty minutes, and so on, so that there is one spectrogram corresponding to the first five minutes of each fifteen minute interval. The positions of the bands are shown graphically by means of the rectangular black blocks with the wavelength scale indicated at the bottom of the chart and the wavelength of each band is given in millimicrons at the left of the block. The first spectrogram for

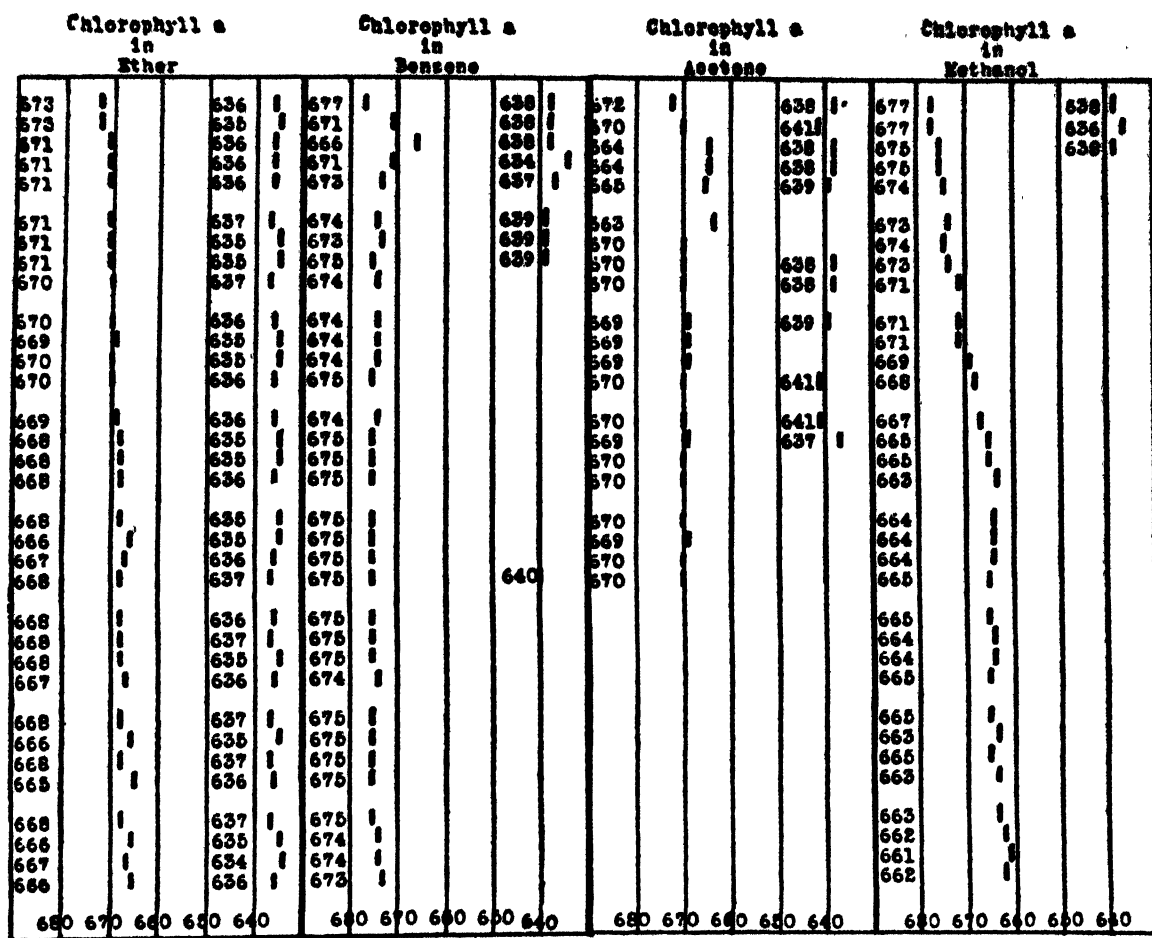


FIGURE 7.

Chart showing graphically the variation in the fluorescence spectra of chlorophyll *a* in the four solvents during the first eight hours of photodecomposition.

each solvent is placed at the top of the chart and the spaces indicate the end of each hour.

It is obvious that in determining the wavelengths of the fluorescence bands it is very important to be sure that no appreciable photodecomposition takes place during the time the fluorescence is being measured or photographed. This factor probably explains some of the disagreement in the values of the wavelengths given by the different observers (Table I). Another factor is the method of determining the positions of the maxima from photographs of the spectra. The value of the wavelength of the band given by Dhéré was made, assuming that there was only one band, by measuring the positions of the same density on each side of the band on the plate and assuming that the position of the band maximum was half way between these two points. While there is considerable disagreement between Dhéré's value and ours, if we measure our densito-

meter curves in the same way that Dhéré measured his plates the values check very well. Dhéré mentions that special precautions were taken to insure no error due to photodecomposition. Zscheile, the only other observer to use pure chlorophyll *a*, measured his intensities point by point with a monochromator and photoelectric cell. This method requires considerable time for a complete observation of the fluorescence spectrum and there is chance for photodecomposition between the beginning and the end of a run. The wavelength obtained by Zscheile corresponds to the value obtained by us after about three hours of photodecomposition with our arcs. It was found in the early part of this investigation that the rate of photodecomposition increased quite rapidly with increase in temperature. For that reason all observations included here were made with the fluorescence tube cooled to five degrees centigrade by circulating an alcohol brine in the

cooling jacket*. Zscheile's observations were made at room temperature.

The densitometer curves for chlorophyll *b* in the four solvents lack the smoothness of those for chlorophyll *a*. The positions of the maxima which appear on these curves have been read from the originals and they show two surprising facts; first, that the positions of these maxima do not change during the photodecomposition process and second, that they are the same in the different solvents. The relative intensities of these individual maxima, however, vary a great deal during the photodecomposition, some increase while others decrease. Table II gives a list of the wavelengths of these bands in the four different solvents. Each of the bands not only appears at the beginning of the run but appears quite consistently at the same wavelength throughout the progress of the photodecomposition.

Table III gives a list of the stronger bands which appear at the beginning for both chlorophyll *a* and *b* and the derivatives which have been studied. These values are all taken from the first exposure for each of the substances. The wavelengths of the principal maxima are indicated by an asterisk. It is interesting to note, in the *a* series, that the band in the neighborhood of 650

m μ does not vary a great deal in wavelength. This is the same band which, for chlorophyll *a*,

TABLE II

Ether	Acetone	Benzene	Methanol
564	564	563	564
568	568	567	568
590	589	590	590
592	592	592	594
596	596	595	597
601	601	602	601
603	603	603	604
607	606	606	606
613	613	612	613
617	615	615	616
620	618	619	619
624	623	623	624
640	639	640	639
647	646	647	646
649	649	649	649
651	652	651	651
670	671	671	670
679	679	680	680
683	682	683	683
685	685	685	685
690	692	692	691
699	699	699	699
709	708	709	708

Table II. Wavelengths in millimicrons of the bands for chlorophyll *b* in the four solvents.

TABLE III

Chlorophyll <i>a</i>	672*					638*				
Methyl chlorophyllide <i>a</i>	683*					643* 628 615 605 597				
Pheophytin <i>a</i>	687*					647* 635				
Methyl pheophorbide <i>a</i>	708	696*	682	671	665	645* 636* 622 611				
Pheophorbide <i>a</i>	683*					664 649*				
Chlorophyll <i>b</i>						657* 637* 619 598 589 585				
Methyl chlorophyllide <i>b</i>	675*					657* 628 596				
Pheophytin <i>b</i>	670*					648*				
Methyl pheophorbide <i>b</i>	689*					669	652*	646*	626	616 608 602 588
Pheophorbide <i>b</i>	683* 679*					652* 623 617 609 606 589				

Table III. Wavelengths in millimicrons of the stronger bands appearing on the first exposures for chlorophyll *a* and *b* and the derivatives studied.

* The alcohol brine was cooled in a Frigidaire water cooler and circulated by means of a constant pressure pump. This pump was kindly loaned to us by Mr. A. E. Berdon.

did not change in wavelength when different solvents were used. Both of the principal maxima for the *b* series vary more in position as we pass through the series than is the case for the *a* series.

By comparing Figs. 3-6 we find that the two principal maxima are, in general, much better re-

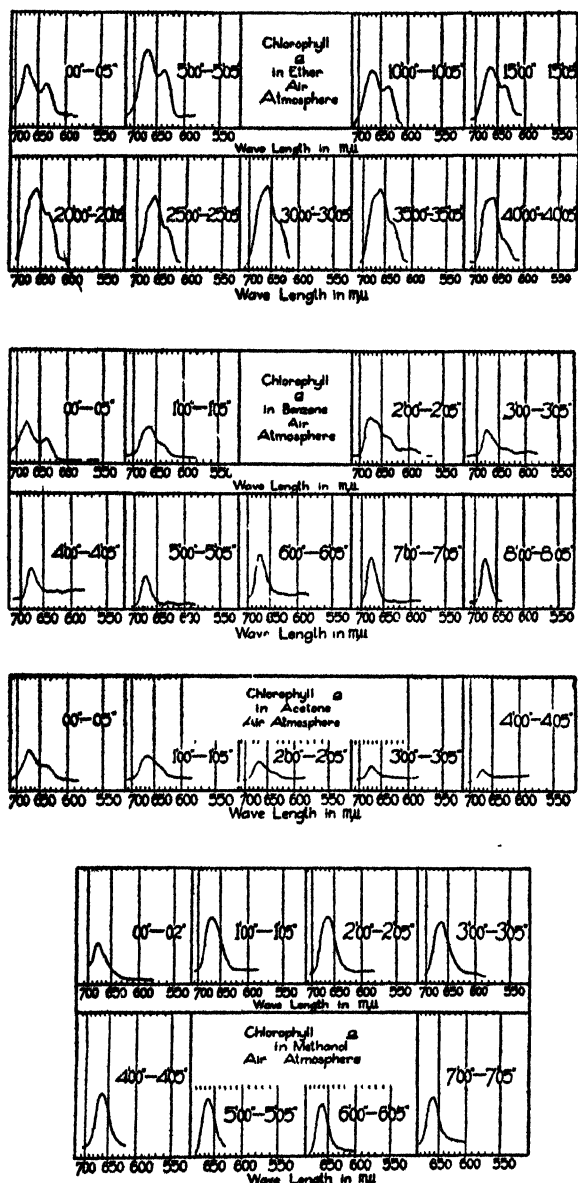


FIGURE 3.

Charts of the densitometer curves of the fluorescence spectra of chlorophyll *a* in the four solvents.

* In all curves of this and the following paper the signs ' and " which conventionally designate minutes and seconds are used to designate hours and minutes respectively.

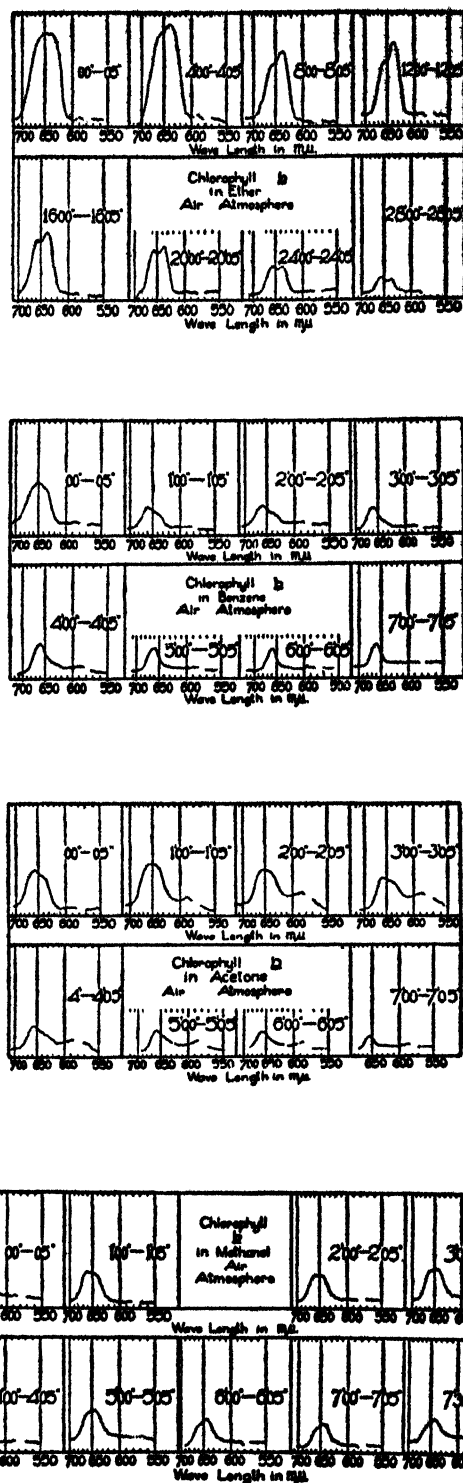


FIGURE 4.

Charts of the densitometer curves of the fluorescence spectra of chlorophyll *b* in the four solvents.

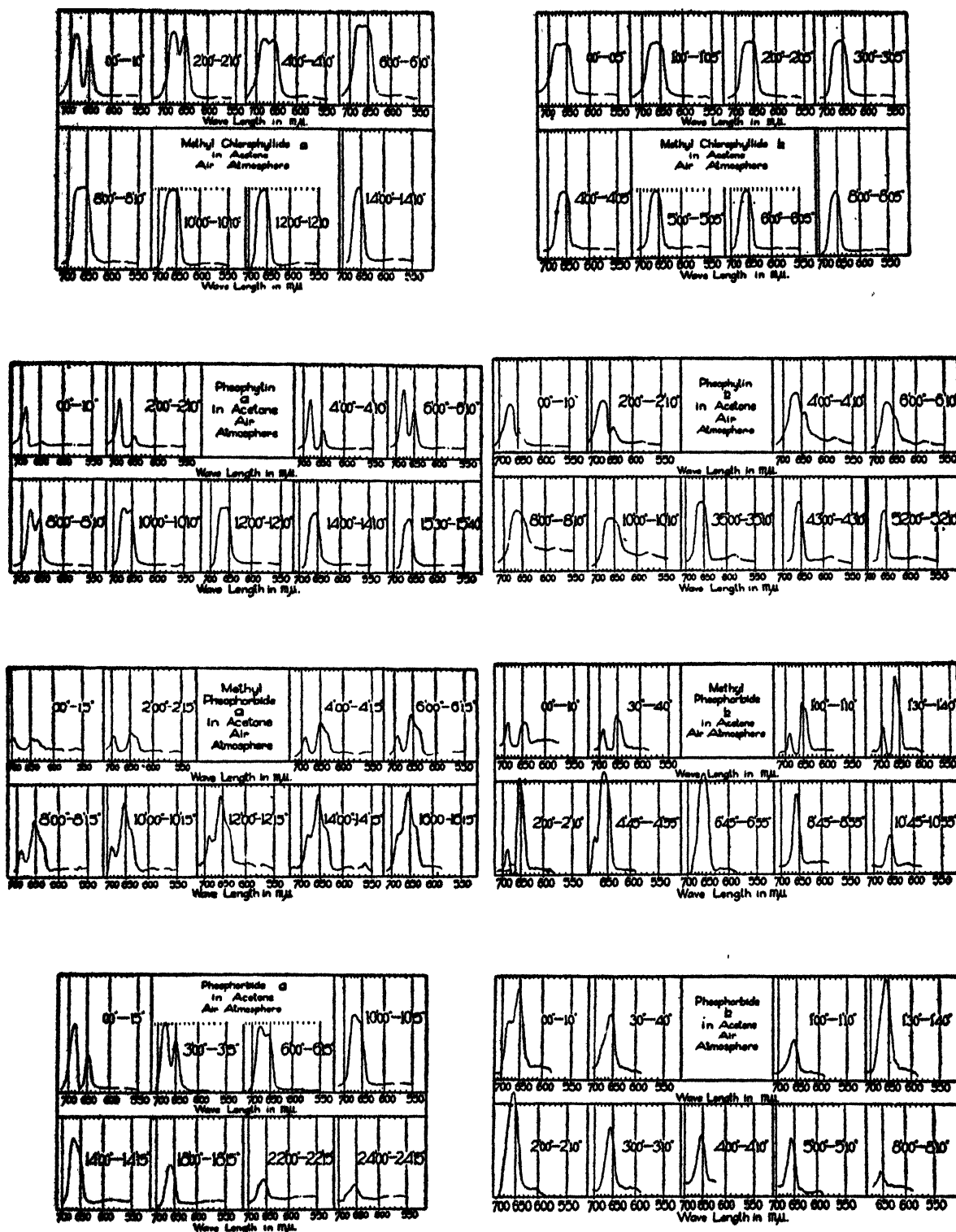


FIGURE 5.

FIGURE 6.

Charts of the densitometer curves of the fluorescence spectra of the four chlorophyll a derivatives in acetone solution.

Charts of the densitometer curves of the fluorescence spectra of the four chlorophyll b derivatives in acetone solution.

solved in the chlorophyll derivatives than they are in the chlorophylls. It is also evident that the band present at the end of a run is nearly always located between the positions of the two principal maxima at the beginning. It is the building up of this band during the process of photodecomposition which undoubtedly accounts for the fact that the two principal maxima become unresolved. The two methyl pheophorbides are the only substances, other than chlorophyll *b*, which show very much structure in their bands. It is found that both of these substances behave like chlorophyll *b*, in that these bands appear at the same wavelengths throughout the photodecomposition, although their relative intensities vary considerably. Table IV gives the wavelengths of these bands for methyl pheophorbide *a* and methyl pheophorbide *b*.

TABLE IV.

Methyl pheophorbide <i>a</i>	Methyl pheophorbide <i>b</i>	
678	687	623
656	685	618
653	658	615
649	655	611
637	635	608
635	633	605
625	631	603
608	629	601
569	627	599
562	625	597

Table IV. Wavelengths in millimicrons of the bands for methyl pheophorbide *a* and *b*.

DISCUSSION OF RESULTS

Although the fluorescence bands for only three of the substances which have been studied show a fine structure, this fact, taken in connection with the behavior of the bands for the other substances, indicates quite conclusively that these bands are not simple but have a fine structure. In most cases the individual bands overlap to such an extent that it is impossible to resolve them under ordinary conditions. In the cases where they have been resolved there is probably considerable error in the determination of the wavelengths because of the overlapping of adjacent bands. The results with chlorophyll *b* in the different solvents indicate that there is no difference in the wavelengths of the individual bands but that the presence of the solvent does have considerable effect on their relative intensities. This change in relative intensities changes the shape of the envelope of the system of bands, thus changing the position of its maximum. The fact that the wavelengths of a large number of individual bands in the case of chlorophyll *b* and

the methyl pheophorbides do not vary during the photodecomposition indicates that the part of the molecule responsible for these bands is not attacked until late in the photodecomposition process. An apparatus is now under construction to be used for studying the fluorescence spectra at liquid air temperature. Under these conditions it is hoped that the bands will be better resolved so that accurate wavelength measurements may be obtained for the individual bands of all of these substances.

In this investigation substances with and without the magnesium atom have been studied, and also substances with the phytyl group present or with it replaced by a methyl group or by an atom of hydrogen. The general character of the fluorescence remains the same in all of the substances. This would indicate that neither the magnesium nor the phytyl group are very closely connected with the part of the molecule responsible for the fluorescence.

An examination of the charts of the fluorescence curves will show that, in general, as the photodecomposition proceeds a band group is developed in the region between the two original groups. The development of this band group and the variation of the relative intensities of the individual bands in the groups are sufficient to account for the rapid shifts which take place in the wavelengths of the principal maxima during the photodecomposition. It is not possible to determine, from the investigation at ordinary temperatures, whether the band group which builds up during the photodecomposition is actually present at the beginning, but a study at low temperatures should clear up this point.

In the early part of this investigation it was found that the rate of photodecomposition increased with increase in temperature. This would indicate that the decomposition is not entirely photochemical. It may be possible to slow down the thermal reactions sufficiently, when very low temperatures are used, so that the photochemical reactions can be separated from the ordinary chemical reactions.

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DISCUSSION

Dr. Mestre: I would like to know a little more about the densitometer used.

Dr. Albers: The spectrograms were densitometered with a projection type densitometer. A densitometer reading was made for each 0.1 mm. along the plate; this distance corresponds to about 1.1 $m\mu$ at 600 $m\mu$.

Dr. Mestre: I would like to ask Dr. Albers whether I am correct in assuming that the curves shown simply represent the densitometer galvanometer readings plotted against wavelength.

Dr. Albers: Yes.

Dr. Mestre: In that case it would seem to me that they cannot be considered to be more than objective evidence that qualitative changes have occurred. Without correction for the characteristics of the photographic plates used it is not possible to state the position of maxima with any precision, and the true form of the fluorescence curve may be very markedly different from that of the densitometer tracing. In my own photographic observations on the absorption spectra of leaves and algae, using a 1 meter concave grating spectrograph giving a dispersion of approximately 15 Å per millimeter, I have found that the red absorption maximum of the densitometer tracing was often as much as 7 $m\mu$ on the long wavelength side of the corrected maximum. With the same plate characteristic and an emission spectrum, the correction would, of course, have been in the opposite direction. It should also be pointed out that without a knowledge of the characteristics of the plate it is not even possible to say in which direction the correction will be.

Dr. Albers: Yes, we recognize that that is true.

Dr. Mestre: There are two other points which I would like to bring up for discussion. The first is the probable extent of effect of reabsorption on the structure of the fluorescence bands. The second is the possible effect of changes in the absorption spectrum, brought about by the photochemical decomposition, on the form and intensity of the fluorescence bands. It would seem to me that it was necessary to determine the changes in the absorption spectrum over the entire spectral region used for irradiation in order to interpret the observed changes in the loci and intensities of the fluorescence bands. If a series of observations could be made using monochromatic excitation of different wavelengths, it would seem to me that much might be learned.

Dr. Albers: We have recognized these points and are planning to make studies of the absorption spectra of these substances during photodecomposition in the near future. We also plan to excite the fluorescence with monochromatic radiation using either a line source with filters or a large monochromator.

Dr. Brackett: I was struck by the same question of reabsorption. Did you make any determination of the transmission in the region, say from 650 $m\mu$ to 670 $m\mu$ in say about 5 cm. path in the medium with the concentration you have used?

Dr. Albers: So far we have made no measurements on the absorption of these solutions so it would be impossible to make any estimates for the transmissions in the region studied. We have noticed, however, that changes in the fluorescence spectra are usually accompanied by changes in the relative intensities of the mercury lines, indicating, in a qualitative way, that the relative transmissions in different regions of the spectrum vary during photodecomposition.

Dr. Brackett: If one chooses such a concentration as would give a maximum total fluorescence, it would be a kind of compromise between emission and absorption, and under those conditions one would have just about the sort of balance between absorption and emission as would explain a good deal of the changes in character observed. With the gradual destruction of material and consequent decrease of absorption one would have that split maximum changing just about in the way indicated.

Dr. Albers: The red absorption band for chlorophyll falls somewhere near the region between the two principal maxima with its center well up on the side of the curve of the long wavelength maximum. Dhéré attributes the minimum in the fluorescence spectrum entirely to reabsorption but

I do not believe that that is correct because of the position of the minimum.

Dr. Brackett: Some of that discrepancy would be taken care of by the plate characteristics which would cause a shift such as that Mestre indicated. In other words, for absorption and emission the shift would be in different directions which might tend to bring the wavelengths into agreement.

Dr. Zscheile: Concerning the chlorophyll components, *a* and *b*, have you used the absorption spectra as the chief criterion of purity?

Dr. Albers: Yes.

Dr. Zscheile: What do you mean by the "normal spectrum" of a chlorophyll component?

Dr. Albers: In the case of the work with the chlorophylls under an air atmosphere the "normal spectrum" of a chlorophyll component is essentially that given by Willstätter.

Dr. Zscheile: Your chlorophyll *b* was undoubtedly impure. I think that may partially account for the much greater complexity of the fluorescence spectrum of the *b* component.

Dr. Albers: Of course, in the case of chlorophyll *b*, all of the work was done with chlorophyll separated by the Willstätter methanol procedure. Chlorophyll *b* prepared by Willstätter's method may contain as much as 10 to 15 per cent chlorophyll *a* according to Winterstein's estimation.

In preparing chlorophyll *b* we were aware of the limitations of Willstätter's method; we therefore subjected our product to repeated Willstätter separation method and used the sample of chlorophyll *b* thus prepared. It did not exhibit the band in the red region of the absorption spectrum characteristic for chlorophyll *a*.

Dr. Zscheile: I have another reason for the disagreement in wavelength of the fluorescence maxima, namely, the method of excitation. You used a line source and I used a continuous source. Your source undoubtedly contained considerable ultraviolet, with the 365 m μ line rather strong, while my continuous source was very weak in the ultraviolet. These different types of excitation would be expected to produce different fluorescence spectra. Although the 365 m μ line may be weakened by transmission through the pyrex, it may excite more intense fluorescence than the visible lines. The fluorescence maxima might be different in relative intensity as well as in wavelength. In regard to the scattered light, when you make an exposure with a tube filled with solvent alone do you get any darkening on the plate?

Dr. Albers: We have no evidence at the present time that the nature of the fluorescence of the chlorophylls is different when different wavelengths are used for its excitation, but there may be a difference in the efficiency of the different

mercury lines for exciting fluorescence. The mercury lines always appear on the spectrograms along with the fluorescence.

Dr. Zscheile: Do your arcs emit considerable continuous radiation in the red region, just where these measurements are being made?

Dr. Albers: As a matter of fact, we have made an exposure for 100 hours with a solution of potassium chlorophyllin in water with no evidence of a continuous spectrum in the scattered light. In the case of the substances studied here, the exposure times were from five to fifteen minutes.

Dr. Zscheile: Can the wavelength shifts in the first fifteen to thirty minutes be consistently reproduced?

Dr. Albers: Yes.

Dr. Zscheile: Many of the bands in the case of chlorophyll *b* are rather close, about 2 m μ apart. What was the slit width in millimeters?

Dr. Albers: We have not measured the slit width. The images of the mercury lines on the plates, however, are narrow compared to the widths of the bands.

Dr. Zscheile: What chemical changes were observed after the whole exposure?

Dr. Albers: So far, we have not made much of a study of the chemical changes in the chlorophylls during photodecomposition, as the concentrations of the solutions used is too low. After the reaction the solution is completely decolorized. Some previous workers have found a positive aldehyde reaction when analyzing the residue after photodecomposition of the chlorophylls, but there is disagreement between these observers on which aldehyde is formed. This observation could be tested by means of studies on solutions of higher concentration. On evaporating a solution of chlorophyll, after photodecomposition, an oily yellow residue is left.

Dr. Zscheile: It would be interesting to know which part of the molecule is attacked (in the first few minutes of exposure) by light of different wavelengths.

Dr. Albers: The series of derivatives studied were selected with the idea of showing whether the presence or absence of the phytyl group or of the Mg played any role in the way in which photodecomposition proceeds. The evidence is that they do not. We have not made any studies on the effect of different wavelengths.

Dr. Rothmund: In the cases of chlorophyll *a* and of pheophytin *a*, the decolorized solutions were evaporated and the oily residues tested with Ehrlich's reagent (p-dimethylamino benzaldehyde). In both cases the tests were negative. The form, in which the magnesium is present after photodecomposition, has not been established.

Dr. Emerson: Would you elaborate a little on the purpose or significance of such a detailed study of the fluorescence changes accompanying the photodecomposition of chlorophyll and its derivatives?

Dr. Albers: The purpose of this study is to learn something more about the properties of the chlorophylls.

Dr. Inman: As to Dr. Emerson's question concerning the significance of such studies, I would like to point out that we know that chlorophylls are integral parts of the photosynthesis mechanism and that they are at least light absorbing molecules. Therefore, any reaction of these molecules to radiation may be relevant to an understanding of what goes on in the normal process of carbon assimilation. This is likewise true for the chemistry of extracted chlorophylls.

Dr. Emerson: You believe that chlorophyll, in carrying on its normal function in the cell, undergoes photodecomposition about which something might be learned through a study of fluorescence of the photodecomposition products outside the cell?

Dr. Albers: I do not know whether chlorophyll decomposes photochemically in the cell in the process of carrying on its normal function or not, but I believe that a knowledge of the properties of chlorophyll, determined with the pure substance, might be of value in determining what the normal function of the chlorophyll is in the cell.

Dr. Inman: I do not necessarily believe that there is photodecomposition of chlorophyll in the living leaf. The fact is, there is insufficient evidence to draw definite conclusions concerning this phase of the mechanism. The studies of fluorescence and photodecomposition represent another approach to a possible explanation of the behavior of the chlorophylls to radiation and may thus contribute something to the explanation of photosynthesis.

Dr. Brackett: I can hardly conceive of a more pertinent approach to the problem of photosyn-

thesis than the investigation of fluorescence. What history we have of the mechanism of the transfers of energy from one molecule to another has hinged very largely on the study of fluorescence. Whether fluorescence is actually present under growing conditions in plants is relatively unimportant. In a study of the general process, undoubtedly if various molecular forms which may occur in the steps involved in photosynthesis are investigated through their fluorescence, one may find some clue to the steps that take place. This is most likely to be found in various types of molecules related to chlorophyll, such as have been discussed.

Dr. Emerson: Is there in your opinion a relationship between carbon dioxide assimilation which is known to take place only in intact cells, and photodecomposition of chlorophyll, which so far as I know has not yet been shown to take place in normal cells, but is characteristic of cells undergoing irreversible injuries, or of chlorophyll extracts?

Dr. Albers: I do not know.

Dr. Mestre: I think that you can say, with a certain amount of safety, that during photosynthesis no photodecomposition of the chlorophyll involving chemical changes of anything like the magnitude considered in this paper are taking place. Photospectrograms of the same leaf, before and after, an extended period of photosynthesis show no change.

Dr. Brackett: One further point in that connection, in the mechanism postulated by Franck two different molecules produce light absorption—"monodehydrochlorophyll"; and secondly, "hydroxy chlorophyll." They might conceivably have different types of fluorescence. It becomes an extremely interesting question whether these exhibit differences in fluorescence and whether these forms exhibit differences when associated or unassociated with carbon dioxide or formic acid. It thus becomes extremely interesting if one can arrive at significant differences.

FLUORESCENCE AND PHOTODECOMPOSITION OF THE CHLOROPHYLLS AND SOME OF THEIR DERIVATIVES UNDER ATMOSPHERES OF O₂, CO, AND N₂

H. V. KNORR AND V. M. ALBERS

INTRODUCTION

It is a well known fact that chlorophyll solutions are rapidly bleached under the action of light. This was first investigated by Senebier in 1782. It has been thought by many investigators that the decolorization is a result of oxidation and that it takes place only in the presence of oxygen. Wurmser¹ concludes that the bleaching of chlorophyll by light is an oxidation process.

Usher and Priestly² state that chlorophyll films, when exposed to sunlight, decomposed CO₂, producing formaldehyde and hydrogen peroxide. They conclude that the hydrogen peroxide is responsible for the bleaching of the chlorophyll in sunlight. This bleaching in sunlight, whether CO₂ is present or not, is due to the formation of hydrogen peroxide. Ewart³, however, showed that chlorophyll films immersed in a hydrogen peroxide solution remained green for a long time when kept in darkness. These same films, if exposed to light, bleached very little faster than similar films exposed to ordinary air.

Wager⁴ showed that bleaching takes place only in the presence of oxygen. He also observed that the rate of bleaching was the same whether CO₂ was present or not. Some question can be raised as to the purity of the chlorophyll used by Wager, as his method of extraction would probably yield the yellow pigments and other substances from the plant as well as the chlorophyll.

Warner⁵ observed that chlorophyll bleached only in the presence of O₂, and that the bleaching took place more rapidly in the presence of water. He considers it probable that the bleaching is due to the hydrogen peroxide, which is formed in the presence of light, since bleaching was observed in the dark in the presence of hydrogen peroxide.

Ewart³ observed that when dry chlorophyll films were exposed to sunlight in nearly dry air, with and without carbon dioxide, the presence of a small amount of CO₂ seemed to accelerate the bleaching. He observed no bleaching with dry chlorophyll films in pure dry nitrogen, even in eight weeks of exposure to light. A slight bleaching was observed when dry CO₂ was used. He also obtained evidence that chlorophyll was able to combine slowly with CO₂ during its oxidation. He exposed thick films of chlorophyll to sunlight in air, containing a little moisture and CO₂ until completely bleached, which required four weeks time. When 0.8 gm. of chlorophyll was allowed to bleach for a month there remained, after exposure, 0.785 gm. by weight. A similar quantity

in pure air lost 68% by weight, and was bleached very much slower. Ewart concludes that CO₂ combines with chlorophyll and forms xanthophyll and a colorless waxy substance; this can take place only in the presence of water and is accelerated by sunlight.

Padoa and Vita⁶ have investigated the effect upon the absorption spectra of chlorophyll *a* and *b*, and the mixture chlorophyll *a* + *b* in solution in benzene, when treated with O₂, CO₂, CO, and N₂. The treatment with the different gases, which extended over a period of several hours, was carried on in darkness, so that very little photodecomposition occurred before the absorption spectrum was obtained. Spectrograms of the absorption and microphotometer tracings were obtained of the chlorophylls *a* and *b* and the mixture *a* + *b*, when treated with the different gases. They observed changes in the positions of the bands, some of the bands disappearing completely. Variation in the relative intensities of the bands was also observed. The absorption spectrum of a solution of chlorophyll *a* + *b*, which had been treated with CO and then treated with O₂ for a long period of time, showed the same absorption spectrum as that treated with O₂ alone. They conclude that this is evidence that CO-chlorophyll compound is a reversible one; that unstable compounds are formed with both of the chlorophylls by O₂, CO₂, CO and that N₂ does not combine with chlorophyll.

The investigations of Albers, Knorr and Rothemund⁷, of the fluorescence of reduced chlorophyll in solution in pyridine under CO₂ and N₂, to be reported by Dr. Rothemund in this volume, showed the importance of a study of the fluorescence and photodecomposition of the chlorophyll solutions under atmospheres other than air.

EXPERIMENTAL

The experimental arrangement used to study the fluorescence and photodecomposition of the various chlorophyll solutions under atmospheres of gases other than air, was the same as that described by Albers and Knorr in the preceding paper, except as modified in order to place the solutions under atmospheres of the various gases.

With the apparatus represented in Fig. 1, it was possible to repeatedly flush the fluorescence tube, with the particular gas to be used, by successive evacuation and refilling. The gas was bubbled through the freshly prepared solution for twenty minutes. The solution was forced into the fluorescence tube and placed under a pressure

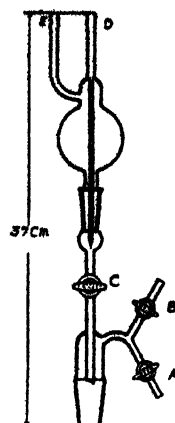


FIGURE 1

of the gas, which was slightly above atmospheric pressure.

DATA AND RESULTS

This investigation included a study of the fluorescence spectrum and the photodecomposition of chlorophyll *a* and the following derivatives, methyl chlorophyllide *a*, pheophytin *a*, methyl pheophorbide *a*, and pheophorbide *a*, in solution in acetone, and under atmospheres of O_2 , CO_2 , and N_2 , respectively. Chlorophyll *b* and its corresponding derivatives were studied under the same conditions.

The densitometer curves, represented in Figs. 2 to 11 inclusive, were obtained in the same manner as those described by Albers and Knorr in

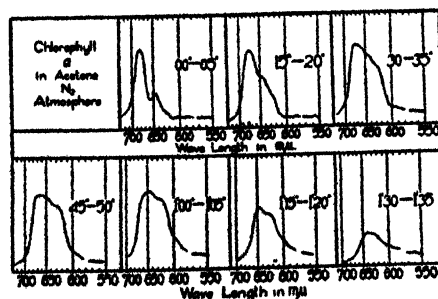
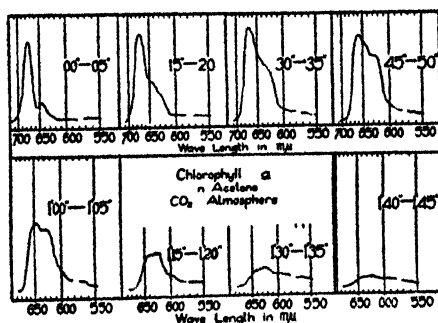
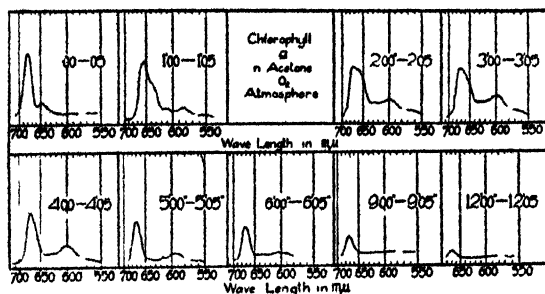


FIGURE 2.

Densitometer curves of the fluorescence spectra of chlorophyll *a* in acetone under O_2 , CO_2 , and N_2 .

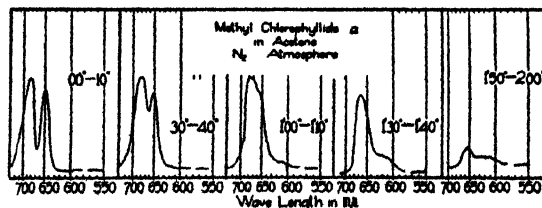
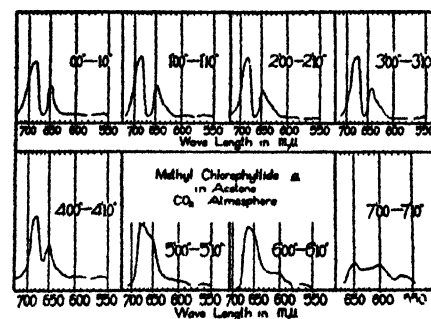
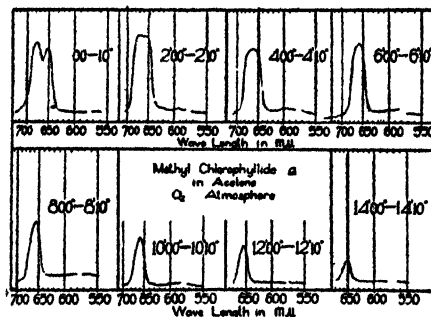


FIGURE 3.

Densitometer curves of the fluorescence spectra of methyl chlorophyllide *a* in acetone under O_2 , CO_2 , and N_2 .

the preceding paper. In each case a few representative curves have been selected to show the variation of the fluorescence spectrum as decomposition and decolorization proceed, in the presence of radiation.

The wavelengths of the principal bands observed during the first exposure, for those substances studied, are represented in tabular form in Fig. 12.

DISCUSSION OF RESULTS

One observes that, in general, the patterns of the spectra, during the first exposure, are the

same for all the substances under the different gases. Each spectrum consists of two or three principal bands in the red region, the separation of the bands varying with the different derivatives. The fact that the fluorescence is not essentially different when the magnesium is removed, would indicate that the role played by this atom is a minor one in this fluorescence. The same reasoning would apply to the phetyl group. One observes that there is very little variation in the position of the shorter wavelength component of this band system for the various substances belonging to the "a series", under the

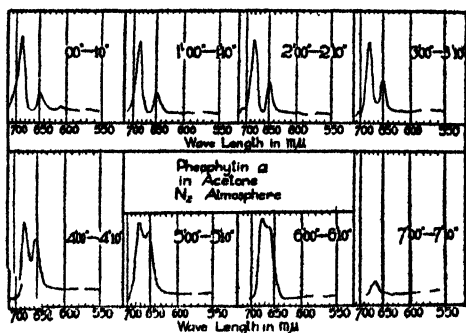
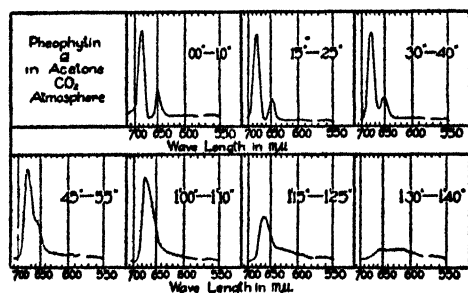
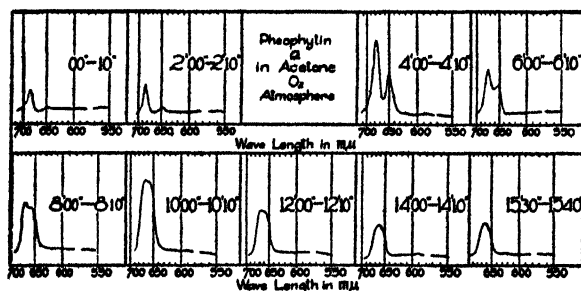


FIGURE 4.

Densitometer curves of the fluorescence spectra of pheophytin a in acetone under O_2 , CO_2 , and N_2 .

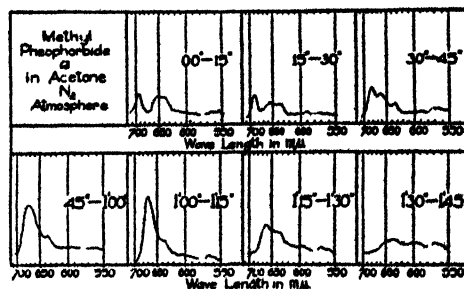
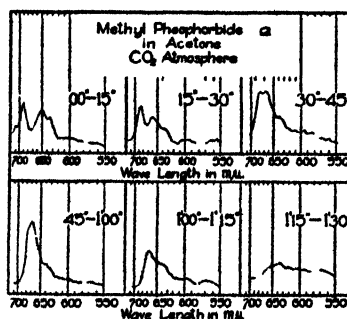
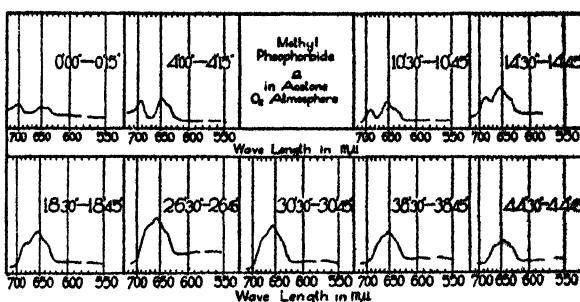


FIGURE 5.

Densitometer curves of the fluorescence spectra of methyl pheophorbide a in acetone under O_2 , CO_2 , and N_2 .

different gases. The position of the longer wavelength component is quite different for the various substances. The separation of the two bands is least for chlorophyll *a* and greatest for methyl pheophorbide *a* and pheophorbide *a*. The fact that positions of the bands during the first exposure are altered by the different gases, would indicate that a more or less intimate combination exists between the substance and the different gases. It seems reasonable to assume that no such relationship would exist in the case of N_2 . There is a difference between the positions of the

longer wavelength component under nitrogen and oxygen in all cases except that of chlorophyll *a*, where the positions of both of the bands are the same under the two gases. The greatest shift in the positions of the bands is observed for methyl pheophorbide *a* under O_2 and methyl chlorophyllide *a* under O_2 .

The relative intensities of the two bands are different for the different substances and also for the same substance under different atmospheres. The relative intensities for the two bands of chlorophyll *a* are nearly the same for all three of the gases. There is a great difference in the actual intensities as well as the relative intensities of the bands of pheophytin *a* and pheophorbide *a* under O_2 and CO_2 . The low intensity of the bands of methyl pheophorbide *a* under all the gases is very striking.

The patterns of the spectra observed during the first exposure for those substances belonging to the "b series" are very similar to those observed for the "a series", consisting of two or three principal maxima in the red region of the spectrum. In the case of chlorophyll *b* only one

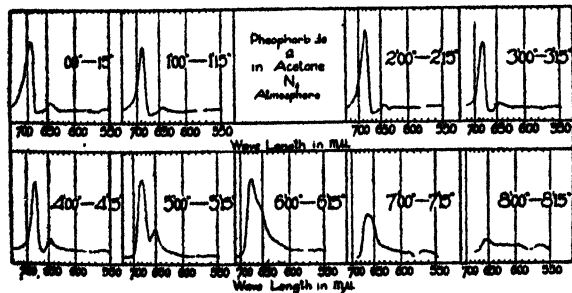
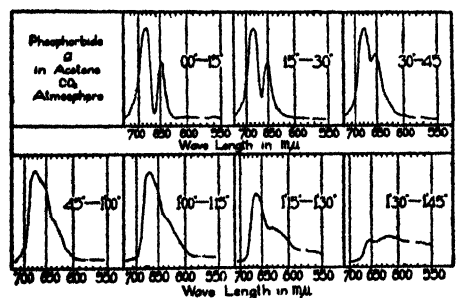
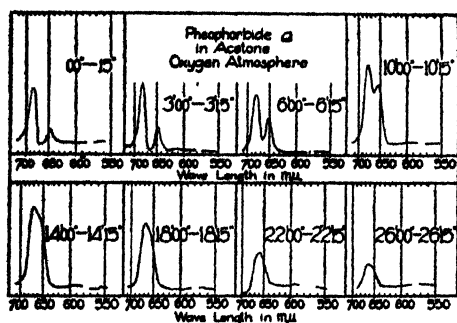


FIGURE 6.

Densitometer curves of the fluorescence spectra of pheophorbide *a* in acetone under O_2 , CO_2 , and N_2 .

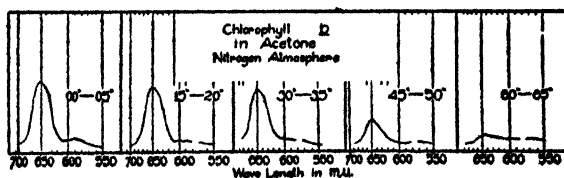
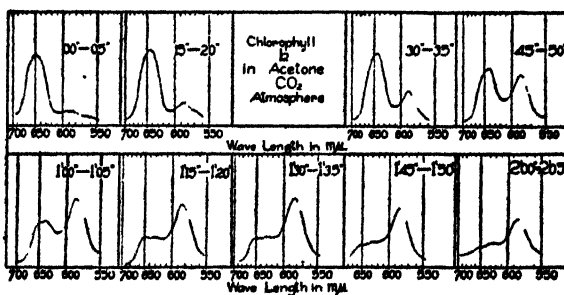
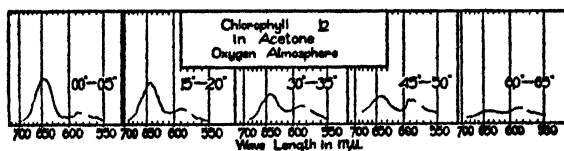


FIGURE 7.

Densitometer curves of the fluorescence spectra of chlorophyll *b* in acetone under O_2 , CO_2 , and N_2 .

maximum is observable. The separation of the maxima for pheophytin *b* and methyl chlorophyllide *b* is less than the separation for the corresponding substances in the *a* series. The positions and the separation of the bands for the methyl pheophorbide *b* differ but little from those of methyl pheophorbide *a* under the different gases. The position of the longer wavelength band under N_2 and O_2 is different in all cases, except that of methyl chlorophyllide *b*, where the positions of both bands under O_2 and N_2 are the

same. The greatest difference in the relative intensities of the two bands occurs in methyl pheophorbide *b* and pheophorbide *b* under all three of the gases. The actual intensity of the principal band of chlorophyll *b* is very nearly the same under each of the gases. The relative intensities of the bands of methyl chlorophyllide *b* are practically the same under all three of the gases. The importance of the relative intensities of the various bands, which appear during the photodecomposition process, must not be overlooked. This is quite evident in the case of the bands with maxima at 587 and 585 $m\mu$. These bands are observed for chlorophyll *b* under CO_2 and O_2 , pheophytin *b* under CO_2 and O_2 , pheophorbide *b* under O_2 . The actual intensity of the bands for chlorophyll *b* under CO_2 is much greater than for any of the other substances.

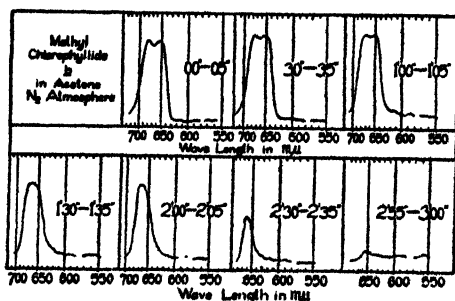
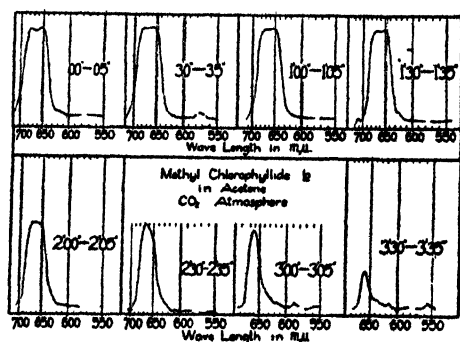
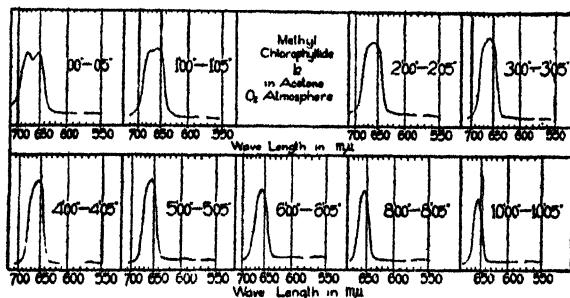


FIGURE 8.

Densitometer curves of the fluorescence spectra of methyl chlorophyllide *b* in acetone under O_2 , CO_2 , and N_2 .

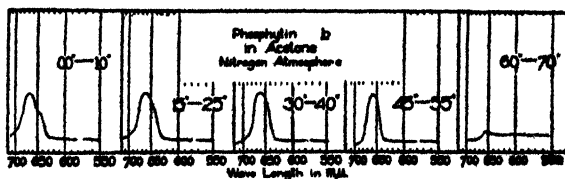
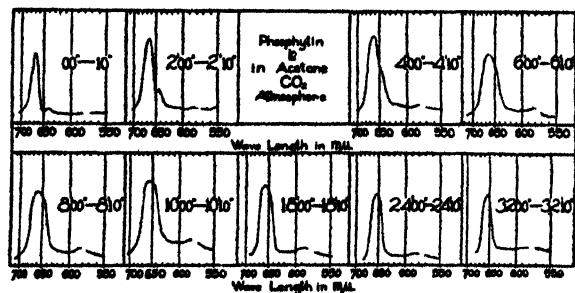
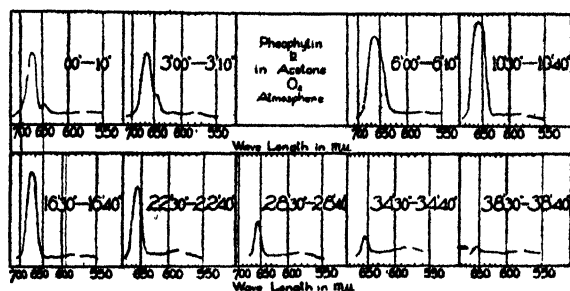


FIGURE 9.

Densitometer curves of the fluorescence spectra of pheophytin *b* in acetone under O_2 , CO_2 , and N_2 .

The fluorescence spectra of methyl pheophorbide *a* under the different gases are more complex than those observed for the other substances. Both pheophorbide *a* and *b* and methyl pheophorbide *a* and *b* show more separate maxima during the first exposure than do the other substances; in this respect their fluorescence spectra are similar to those observed by Dhéré⁸ for some of the porphyrins in this same region of the spectrum.

The well known inhibiting influence of oxygen upon photochemical reactions can be inferred from the behavior of the various substances un-

der an oxygen atmosphere during the photodecomposition process. The time required to decompose the solutions of the different substances under the oxygen atmosphere was much longer than the time required for the same substance under a nitrogen atmosphere. Chlorophyll *b* is the only exception, and here the times were the same.

The statement of Kautsky⁹ that oxygen in the solutions of chlorophyll destroys the fluorescence is not verified by these experiments. The intensity of the fluorescence is quite high during the first exposure for all the substances except methyl pheophorbide *a* and *b*. The time during which these substances fluoresce is far longer under O_2 than under CO_2 or N_2 .

The various atmospheres undoubtedly have an important influence on the velocity of the photo-

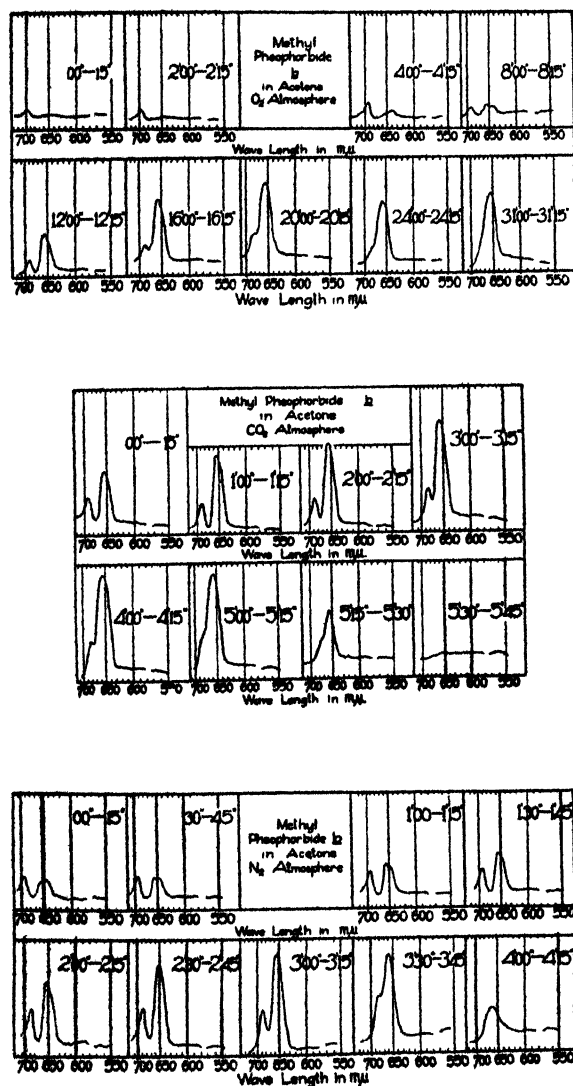


FIGURE 10.

Densitometer curves of the fluorescence spectra of methyl pheophorbide *b* in acetone under O_2 , CO_2 , and N_2 .

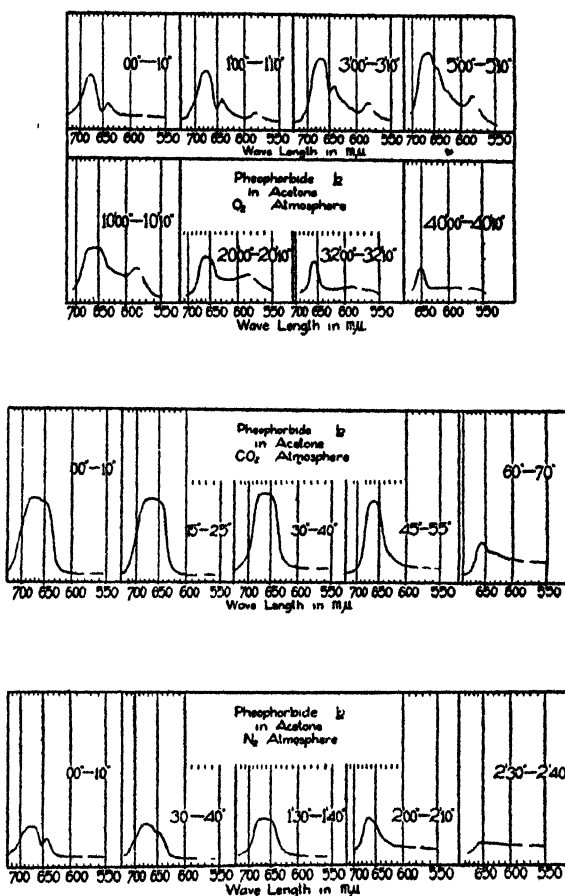


FIGURE 11.

Densitometer curves of the fluorescence spectra of pheophorbide *b* in acetone under O_2 , CO_2 , and N_2 .

TABLE I.
"A Series"

	Chlorophyll	Methyl Chlorophyllide	Pheophytin	Methyl Pheophorbide	Pheophorbide
O ₂			685	697	684
	679	678			
	647	652	646	652	647
CO ₂				637	
	677	685	683	689	683
	646	649	650	652	649
N ₂				636	
	679	683	687	692	688
	648	650	649	651	646
				637	

TABLE II.
"B Series"

	Chlorophyll	Methyl Chlorophyllide	Pheophytin	Methyl Pheophorbide	Pheophorbide
O ₂				695	
		679	668		676
	646	655	646		642
CO ₂				692	
		676	667		681 673 664 652
	649	654	643	657	
N ₂				693	690 677 669
	651	656	665	656	
			646	648	643

FIGURE 12

Wavelengths in millimicrons of the principal bands observed during the first exposure.

chemical reactions which occur during the decomposition process. Likewise they must play an important role in determining the direction in which a particular reaction will proceed. This effect is very noticeable in the behavior of chlorophyll *b* under CO₂, whose spectrum presents by far the most spectacular appearance of any of the spectra studied in this investigation. The bands with maxima located at 587 and 585 mμ appear after the first fifteen minutes exposure, they continue to increase in intensity as the exposure continues, reaching a maximum of intensity after one hour and thirty minutes, and then decrease and vanish after two hours and fifteen minutes of exposure. A similar phenomenon is observed in the case of chlorophyll *a* under oxygen, where a new band appears in the region of 596 mμ after two hours of exposure.

It is obvious from the foregoing data, that the chlorophylls and the derivatives studied in this investigation in acetone solutions, do decompose in the presence of O₂, CO₂, and N₂, when exposed to the radiation of a mercury arc.

It is quite evident from the data presented in this and the preceding paper that the need for more accurate wavelength determinations and some quantitative measurements of the intensities of the bands is exceedingly great. The effect of the solvents, that do not contain oxygen, is now being investigated and we hope thereby to obtain more information concerning the bleaching of chlorophyll under the action of light.

SUMMARY

1. The patterns of the spectra, for those substances studied under the conditions of this investigation, are very similar.
2. The fluorescence spectra of the chlorophylls and their derivatives in acetone solution and under atmospheres of O₂, CO₂, and N₂, possess a complex structure.
3. The fluorescence vanishes as the solutions are bleached.
4. The chlorophylls and the derivatives studied do decompose, when in acetone solutions and under atmospheres of O₂, CO₂, and N₂, under the action of light.
5. The relative intensities of the individual bands change considerably during photodecomposition, but the positions of the bands do not shift.
6. The times required for photodecomposition vary many fold for the different substances when under the various atmospheres.

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DISCUSSION

Dr. Noyes: While the interesting data of Knorr and Albers indicate essential differences in the fluorescent behaviors of chlorophyll *a* and *b* and their derivatives, it seems difficult to draw quantitative conclusions from these experiments. In the first place, acetone itself is far from an inert substance. A sensitized polymerization or oxidation of acetone is not beyond the range of possibility, thereby producing substances which would quench the fluorescence or react with some of the compounds studied. It is difficult, unambiguously, to ascribe the change in fluorescence, therefore, either to a change in the starting molecules or to a primary reaction between them and any of the gases investigated.

Studies of this type to be quantitative should involve a change in the partial pressure of the various gases, and a measurement of the variation of fluorescent intensity with incident intensity and temperature. Above all an inert solvent, preferably one that is completely saturated, would be advisable. Only then could one be sure that the strengthening of one band relative to another is due to some essential change in the molecule of chlorophyll or its derivative. The conclusion is inescapable that these molecules do disappear during irradiation, but the mechanism is far from clear and is undoubtedly complex. If the fluorescence is really a measure of the concentration of the original molecules, it should be possible to arrive at a quantitative (although perhaps empirical) expression for the rate of reaction by such studies.

The fact that pheophorbide *a* and *b* fluoresce for longer under O₂ than under CO₂ or N₂ may indicate some sort of a photochemical reduction (perhaps involving acetone) which is inhibited by oxygen. This point should be tested further.

Dr. Knorr: The discussion by Noyes is very appropriate. Experiments using oxygen free solvents are now in progress. It is planned to carry on these same investigations at low temperatures and reduced partial pressures; in this continuation of our work we hope to follow up the quantitative side of the problem.

Dr. Burk: Have you any opinion at all whether any CO₂ compound forms, or whether CO₂ acts indirectly, perhaps in any of a number of possible ways?

Dr. Knorr: In the case of chlorophyll *b* under CO_2 , where the behavior is quite different, I think there was an intimate combination. Whether or not it combines in a stoichiometric ratio, I am unable to say. In the case of chlorophyll *a* no such evidence has been found as yet.

Dr. Burk: Is there any reason, however, for supposing that chlorophyll *b* might form a compound with CO_2 more readily than chlorophyll *a*?

Dr. Knorr: I have no reason for supposing this. I am simply basing my opinion on the spectra during the decomposition under CO_2 .

Dr. Burk: Would there, then, not be quite a number of other possibilities for the indirect influence of CO_2 ?

Dr. Knorr: Yes. Since the number of possible indirect influences is large, it will be difficult to differentiate between them. Further studies with different solvents might throw some light on that subject.

Dr. Emerson: How do you account for the observations quoted from other workers who found that photodecomposition of chlorophyll requires gaseous oxygen? Your results evidently are not in agreement with this.

Dr. Knorr: Our experiments with solutions are not in agreement with those on dry film made by others.

Dr. Emerson: It seems to me you go a little beyond the evidence when you say that the longer duration of fluorescence in oxygen is due to inhibition of photochemical actions by oxygen. Your measurements show disappearance of fluorescence, but it is not clear from what you have said, that cessation of fluorescence is necessarily coincident with cessation of photochemical activity. Considering the lack of information concerning the chemical changes taking place during bleaching, I doubt if the duration of fluorescence gives any indication of either the extent, or the duration, of photochemical action.

Dr. Knorr: There can be no question of the fact that since the solutions are decolorized under the action of light there must have been one or more photochemical reactions during the process. We observed that under the O_2 atmosphere the time required for decolorization was much longer than when N_2 was used, and as stated in the text, when the fluorescence ceased the solutions were colorless. These observations are in agreement with the fact that oxygen is an inhibitor of these photochemical reactions. One can say with certainty that the rate of decolorization was retarded by the O_2 atmosphere.

Dr. Brackett: Am I correct in assuming that you are dealing with a closed system—no oxygen supplied?

Dr. Knorr: The system was closed during the entire exposure.

Dr. Brackett: Would you notice pressure change in the system?

Dr. Knorr: Not with our present set-up.

Dr. Brackett: That raises the question—frequently reactions take place in the presence of ultraviolet radiation or with visible radiation together with a sensitizer, in which the solvent takes up oxygen due to a photochemical reaction, and consequently the system is being purged of free oxygen. This would account for the gradual rise of fluorescence in the case where an oxygen atmosphere was used.

Dr. Knorr: That is possible.

Dr. Rollefson: From the evidence in these experiments there are at least two reactions involved. First, one causing the change in character of the fluorescence; second, the reaction causing a decrease in the intensity of the fluorescence. It looks as if it might fit a first order law. Have you ever tried to fit a rate law to the decrease in fluorescence?

Dr. Knorr: No.

Dr. Mestre: Such calculations are not yet possible as these data are not quantitative as to intensity of the fluorescence.

Dr. Rollefson: All that is necessary is the determination of the characteristics of the plate.

Dr. Mestre: Calculations might be made from the experimental data in this way, but they would not be very accurate as the γ of the calibration plates might differ from those used and their wavelength characteristics might also not be the same. As mentioned in the discussion of Albers' paper, correction for the plate characteristic may change the form of those curves very much. I would not be surprised if some longer wavelength fluorescence bands would fail to come out if this correction was made, as the plate sensitivity is falling off very rapidly in this region.

Dr. Knorr: This is particularly true in the case of the pheophorbides where the band is much farther toward longer wavelength limit of sensitivity of the plate. The important thing is that the apparent form of the band changes from one exposure to the next.

Dr. Rothemund: I would like to say a few words in regard to Rollefson's question about the order of the reaction. Phytol was never found among the decomposition products. We would have liked to determine first the amount of phytol in the reaction mixture, as this determination would have offered a possibility of determining the order of the disintegration reaction.

One evidence for the photo-oxidative character of the reaction is the fact that the oily residue after the evaporation of the solvent exhibits nega-

tive Ehrlich reaction; when the residue is heated to 124° centigrade *in vacuo*, a sublimate of white crystals is obtained. These crystals suggest a maleic acid derivative and are now under investigation.

Dr. Mestre: I noticed that in the slide in which you showed the spectrograms of chlorophyll *b*, the intensity of the mercury lines was increasing steadily from first to last exposure. Was this due to changes in the intensity of the arc?

Dr. Knorr: It was due to progressive change in the absorption of the solution. The arcs were operated for some time before the exposure was made and had sufficient time to come to equilibrium.

Dr. Mestre: Do not the densitometer tracings include some of the mercury lines, thus complicating the determination of the position and structure of the shorter wavelength fluorescence bands considerably?

Dr. Knorr: Yes, the yellow and green lines are included. Obviously these are complicating factors, particularly in the bands located at 585 and 587 $m\mu$, because these are affected by the unmodified scattering in the lines. Nothing was done to correct that. It makes the reading of the positions questionable.

Dr. Brackett: Have you ever observed a case in which the fluorescent material forms a flocculent precipitate with the material thus going out

of solution? One would then observe a sudden burst of the unmodified lines and disappearance of the general absorption. I have had that experience working with sensitizers on photographic plates.

Dr. Knorr: I think we had one example: reduced chlorophyll in pyridine under CO_2 .

Dr. Emerson: You attach special importance to the unusual behavior of the fluorescence bands of chlorophyll *b* in carbon dioxide. If this has any relationship to a possible chlorophyll-carbon dioxide compound in photosynthesis, one might reasonably expect such behaviour to show up at carbon dioxide concentrations much lower than one atmosphere.

Dr. Knorr: The same bands located at 585 and 587 $m\mu$ were also observed when the chlorophyll *b* solutions were in the presence of air, but the intensities of these bands were much weaker than when an atmosphere of CO_2 was used. Apparently the concentration of CO_2 only affected the intensities of the bands.

Dr. French: Did you take all oxygen out of the nitrogen and carbon dioxide?

Dr. Knorr: The oxygen was removed from the nitrogen by bubbling it through a fresh alkaline-pyrogallol solution. Luminescent bacteria did not luminesce in this nitrogen. The CO_2 from a Kipp generator was bubbled through sodium carbonate solution. The oxygen was bubbled through KOH solution.

TOWARD A MORE QUANTITATIVE PHOTOCHEMICAL STUDY OF THE PLANT CELL'S PHOTOSYNTHETIC SYSTEM

F. PAUL ZSCHEILE, JR.

When the various techniques employed in the study of photosynthesis as a physiological problem are surveyed, the scanty consideration of the exact measurement of the quantity and spectral quality of radiation employed is immediately apparent. This is also true when the photochemical and optical properties of the pigments of the green leaf are considered. On the other hand, much well-warranted effort has been expended on problems that involve molecular structure of the leaf pigments and upon rate measurements of certain physical and chemical steps of the general photosynthetic reaction. The steps most accurately studied have been those involving as principal factors, processes such as diffusion and enzymatic "dark reactions", which also occur in other systems more readily adapted to quantitative control. Since the more purely photochemical steps of photosynthesis, if not the most important, are surely the reactions unique to photosynthesis, it seems that these processes deserve considerably more quantitative attention.

Pigment analyses of plant tissues have heretofore been too inclusive and the various components, or individual chemical substances composing the pigment groups, as the chlorophylls and carotenes, for instance, have been analyzed usually as groups. The methods used have been such that appreciable variations in component ratios would cause serious error in the total pigment content, while the variations themselves would not even be detected, much less measured. The problem of pigment differentiation in analysis of plant tissues may be most satisfactorily solved by the spectro-photoelectric analytical method. Instead of analyzing for total chlorophyll or total carotenoid content, solutions may now be analyzed for the separate components of these pigment groups. This may be done in a strictly objective manner, more quickly and more accurately than with the chemical, colorimetric, and photographic methods in use at the present time. When the optical properties of the various individual pigment compounds have been studied further, the effect of environmental conditions upon their ratios may be accurately measured and such determinations will probably result in a more exact knowledge of their individual functions in the cell's metabolism.

Discussion of a spectro-photoelectric technique

Various features of an apparatus designed primarily for the accurate measurement of low intensities of radiation confined to narrow spectral

regions will now be discussed. It is hoped that this technique will be useful in the solution of photosynthesis problems in which a very accurate measurement of the quantity of radiant energy employed and an exact knowledge of its spectral quality are necessary. Its biological applications will be discussed later.

The apparatus* was designed for quantitative measurement of absorption spectra of substances in solution. The average working intensities of light used for this purpose are of the order of 10^{-8} lumens, considerably lower than those usually used in absorption spectra measurements as a part of photosynthetic studies. This is equivalent to the luminous flux per square cm. from a candle 100 meters distant. This is accomplished by amplification of the small photoelectric current of the order of 10^{-18} amps. A specially-made caesium-caesium oxide photoelectric cell, with a plane quartz window, is sensitive throughout the entire ultraviolet and visible regions and in the near infrared. Its sensitivity at 90 volts is about 4.4×10^{-5} amps. per lumen per cm^2 . It is calculated that the system could detect a candle at a distance of about one mile.

A modified DuBridge and Brown circuit⁽¹⁾, with an FP-54 Photron tube, is sufficiently stable to permit the measurement of light intensities at 10 second intervals with a precision of 1 part per 1000 and the determination of absorption coefficients at 30 second intervals with an instrumental error of only $\pm 0.3\%$. A stable voltage sensitivity of 175,000 mmi. per volt and a current sensitivity of 1.25×10^{16} amps. per mmi. at 10 meters scale-to-telescope distance are the usual sensitivities. The galvanometer period may be decreased to 7 seconds by critical adjustment of the circuit. A 4.5×10^{16} ohms resistance connects the tube grid to the ground.

A narrow spectral region is isolated from a continuous spectrum by a Zeiss Fixed Arm Spectroscope (with deviation of 90°) used as a monochromator. This instrument, designed for the study of weak radiation, such as fluorescence, is especially adapted to this problem. Its lenses have an aperture ratio of F/5. The effective aperture ratio of the entire instrument is F/6.4, referred to $\lambda 4861 \text{ \AA}$. The amount of scattered radiation is negligible. This is shown by exper-

* Constructed at the University of Chicago, with the aid of a grant from the Rockefeller Foundation.

(1) DuBridge, L. A. and Brown, H., *Rev. Scientific Instru.*, **4**, 532, 1933.

iments with a mercury arc. When the 4047 Å. line was in sharp focus, with .01 mm. slits, a maximum galvanometer deflection of 85 cm. was obtained. The wavelength drum was rotated 3 Å. and the deflection was certainly less than 0.5 mm., for it could not be detected. In the same way, with .01 mm. slits, deflections of 85 cm. decreased to 0.0 cm. when the wavelength drum was moved 4 Å. away from the 4358 Å. reading and 45 cm. deflections decreased to 0.0 cm. when the drum was moved 5 Å. from the 5790 Å. reading. At 4358 Å. the scattered radiation is less than 0.1% of the total.

This monochromator, combined with the above-described intensity-measuring system and suitable cell carriages, lenses and light sources, constitutes a spectro-photoelectric apparatus of high accuracy and precision and wide adaptability.

A suitable light source for absorption spectra work in the visible and near infrared regions is an automobile headlight bulb, operated on batteries. A hydrogen arc is used as a source of steady continuous ultraviolet radiation.

Very important items in this type of spectroscopic work are a comprehensive calibration of the entire apparatus and a complete knowledge of errors and limitations. These have been neglected too often in the literature and this fact has made the results of many pieces of research so incomparable with each other that one does not serve to check the other but merely adds confusion to another series of inaccurate observations. For adequate comparison between spectra measured by photoelectric methods, it is imperative that the spectral region isolated be accurately stated. This region must often be exactly the same in the two cases before differences of measuring systems may be dismissed and other more

interesting problems of chemical purity, source, constitution, etc., of the samples under examination can be considered. This last point, concerning exact equality of isolated spectral regions, is more important when absorption maxima are sharp and narrow than in the cases of broad diffuse bands.

To determine the spectral region isolated by the apparatus under discussion, several determinations and calculations are necessary. A steady mercury arc is convenient as a source of several well-isolated lines distributed in the visible and ultraviolet regions. Intensity measurements are made at small intervals as the image of the first slit of the monochromator is passed over the second slit by rotation of the prism. The maximum emission value is obtained by alternate adjustment of prism and collimator lens. In the Zeiss instrument, the lenses are achromatic. Nevertheless, a slight adjustment is necessary to correct for small residual chromatic errors. Curvature of the image of slit 1 on slit 2 is made negligible by decreasing the height of slit 1 to 2 mm. When the line is critically focused, λ is plotted against intensity and a sharp, symmetrical peak results. After a number of lines have been plotted thus, the wavelength calibration curve for the wavelength drum is easily drawn. The accuracy of the wavelength setting is ± 1 Å. for blue and ± 3 Å. for red light. The half-intensity width of the curve for each line, expressed in Å., is then correlated with slit width as measured in mm. to obtain the dispersion, or slit width in terms of Å. per mm. slit width. The dispersion may be most accurately determined by photography of a line spectrum with the plate in the focal plane of slit 2. When a source of continuous light is then used, slit widths will vary with the wavelength, inten-

TABLE I. *Slit Data. Visible Region (50 c.p. Mazda lamp).*

λ	Dispersion Å./mm.	Region Isolated Å./mm. slit	Practical Slit Widths for Absorption Spectra	Calculated Regions Isolated	Deflection
4000 Å.	52	156	0.03 mm.	4.7 Å.	50 cm.
4150	60	180	.02	3.6	55
4300	67	201	.015	3.0	50
5000	116	346	.004	1.4	100
6000	225	675	.002	1.35	100
7000	420	1260	.002	2.5	100

TABLE II. *Slit Data. Ultraviolet Region-Hydrogen Arc. Solvent C₂H₅OH (Cell Length 2 cm.)*

λ	Dispersion Å./mm.	Region Isolated Å./mm. slit	Practical Slit Widths for Absorption Spectra	Calculated Regions Isolated	Deflection
2150 Å.	20	60	0.30 mm.	18 Å.	12 cm.
2300	26	78	.20	16	60
2400	33	99	.15	15	75
2450	34	102	.10	10.2	50
2550	41	123	.07	8.6	45
2750	55	165	.07	11.5	69
2900	68	204	.06	12.2	66
3400	124	372	.04	15	76
4000	207	621	.04	25	66

sity and emission characteristics of the source, sensitivity of the photoelectric cell, sensitivity of the amplifying system, efficiency of the accessory optical parts, and degree of precision desired. However, from the original calibration data, the slit width at any particular wavelength may be calculated easily in terms of Å. From simple geometrical considerations, the spectral region isolated, expressing the wavelength limits of the radiation emitted from the second slit of the monochromator, is then three times the slit width, as expressed in Å. The greater part of the radiation is of the wavelengths of the central half of this isolated region and the fractional intensity rapidly decreases to zero as the limiting wavelengths are approached. To demonstrate the performance of the instrument slits of .002 to .03 mm. may be used throughout the region from 4000 Å. to 7000 Å. and they isolate spectral regions varying from 2 to 4 Å in width.

Application to problems of photosynthesis

Next I shall discuss the application of this experimental equipment to problems of photosynthesis. Of all the leaf pigments, probably the carotenoids have been studied spectroscopically more accurately by more individual workers and under more varying conditions than have the chlorophylls or any other group. In the early stages of the development of this apparatus⁽²⁾, when the measuring system was less sensitive, the

spectra of chlorophylls *a* and *b* were determined in ether solution⁽³⁾. These spectra are given in Fig. 1. The method was sufficiently accurate at that time to demonstrate that components *a* and *b* had not previously been isolated completely from each other and that, in this case, the spectro-photoelectric method is far more sensitive for the detection of impurities than any method in use. Such a test method would be useful as an aid in the purification of any compound having continuous and characteristic absorption in the spectral regions available for study.

In ether, in which color differences between the chlorophyll components are most evident, the state of the pigment is far different from that in the green leaf. It would be of great assistance to have accurate measurements of the absorption spectra of the green and yellow plant pigments in all of the common solvents as a general background for a similar study in such fats and oils as may be associated with them in the living leaf. Even though chlorophyll absorbs light to some extent at all wavelengths, all may not be utilized in photosynthesis. Before accurate and extensive experiments could be made on living material to investigate this point, quantitative absorption data must be available. Many of the experiments on quantum efficiencies and energy relations of photosynthesis are limited in their scope because of the fact that a line source of radiation was used. Such sources are excellent for certain ex-

(2) Zschelle, F. P. Jr., Hogness, T. R., and Young, T. F. *J. Phys. Chem.*, **38**, 1, 1934.

(3) Zschelle, F. Paul, Jr. *Bot. Gaz.*, **95**, 529, 1934.

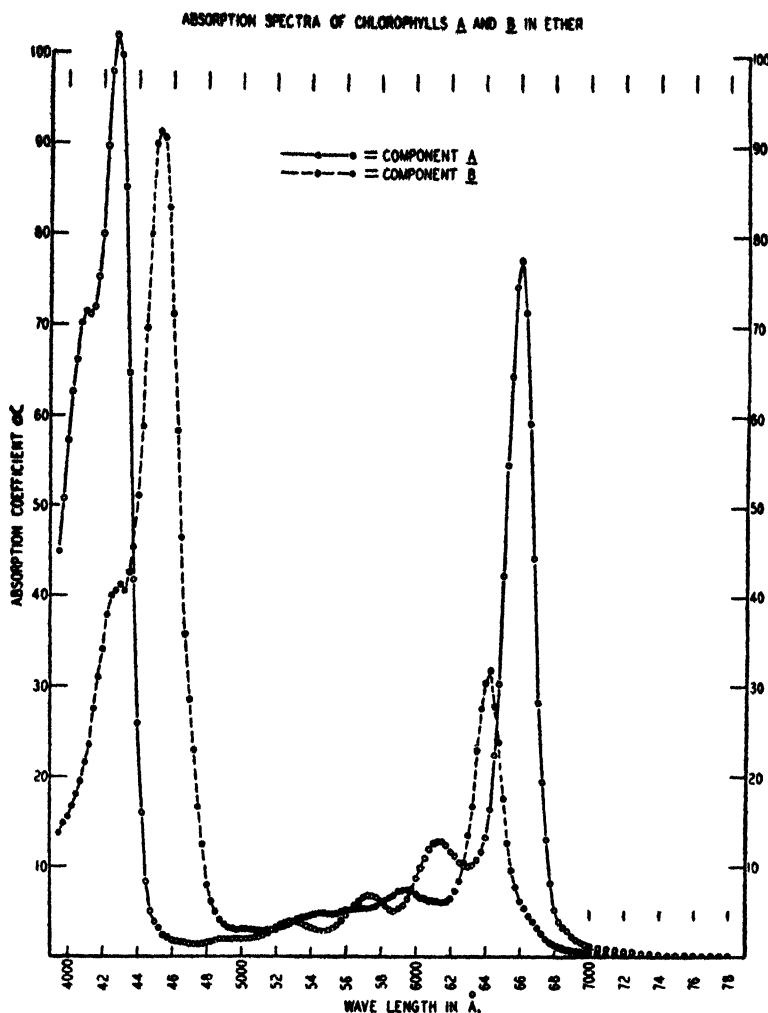


FIGURE I.*

periments but it is accidental if the wavelength of any particular line coincides with that of the absorption maximum of the pigment studied. Filters are very poor for the isolation of lines. It would be a great advantage to be able to select any narrow region (of a few Å.) from the spectrum of a continuous source. Reflection data would also be advantageous.

The recent studies by Kautsky and co-workers⁽⁴⁾, on the fluorescence of leaves under different conditions, indicate strongly that the fluorescent properties of chlorophyll are intimately

associated with the absorption of radiant energy and its conversion into chemical energy as one part of photosynthesis. If their exciting source had been visible light rather than ultraviolet, their experiments would have more physiological significance. This apparatus is well adapted to fluorescence studies, particularly to the quantitative spectral analysis of fluorescence radiation from chlorophyll. This was shown by experiments⁽⁵⁾ with the earlier method of photoelectric current measurement. In ether solution, chlorophyll *a* has two fluorescence bands and chlorophyll *b* has three bands in the region 6300-8200 Å, as shown in Fig. II. The present apparatus would permit the use of much narrower slits and these bands would probably appear sharper. Further experiments might show bands at lower wavelengths.

* Reprinted from an article on chlorophyll, *Botanical Gazette*, Vol. XCV, No. 4, 529 (1934).

(4) Kautsky, H., Hirsh, A., and Davidshofer, F. *Ber.*, 65, 1762, 1932.

Kautsky, H. and Hirsh, A. *Biochem. Z.*, 274, 423, 1934.

Kautsky, H. and Spohn, H. *Biochem. Z.*, 274, 435, 1934.

(5) Zscheile, F. P. Jr. *Protoplasma*, 22, 513, 1935.

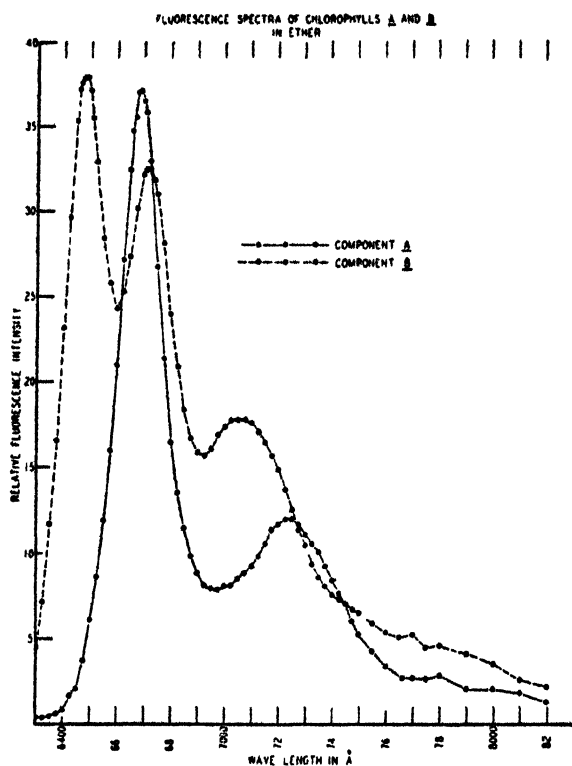


FIGURE II.*

The method of quantitative analysis by the determination of absorption coefficients of mixtures of pigments at specific wavelengths has been used successfully by Zscheile⁽⁶⁾ on mixtures of pure components *a* and *b* of chlorophyll and by Miller⁽⁷⁾ on binary mixtures of alpha and beta carotene, beta carotene and lycopene, and lycopene and leaf xanthophyll. Miller⁽⁸⁾ also applied it to plant extracts containing two yellow pigments. It is necessary, of course, to determine very accurately the absorption coefficients of the pure substances involved at the wavelength employed. For highly accurate results, the solutions must obey Beer's law over the concentration range studied. If Beer's law were not obeyed, the lines of Fig. III would not be straight. Mixtures of chlorophylls *a* and *b* in 90% acetone, made up by weight and dilution, were analyzed with an error of $\pm 1.0\%$ or less (in percentage composition), using the average of two wavelengths in the blue region where the absorption bands are rather steep. Known binary mixtures of alpha and beta

carotene were analyzed with an error of $\pm 0.2\%$ or less (in percentage composition), using the average of three wavelengths.

When compared with visual and photographic absorption spectra measurements, and to chemical methods as analytical tools for the determination of the plant pigments, the advantages of the spectro-photoelectric method are at once apparent. It does not have the subjective errors of visual colorimetric and some photographic methods, nor does it involve drastic chemical changes of the molecules whose content in the original material is the object of the analysis. These are serious objections to the methods now in use for chlorophyll determination. All that is needed is a quantitative extraction of the pigment from the leaf with suitable solvents. Perhaps a simple fractionation would be necessary in some cases in which other pigments might interfere. These are problems for further experimentation. This method also has the advantage of speed, in as much as observations requiring only a few minutes will give the data from which the pigment composition of the mixture may be calculated. Undoubtedly the method can be extended to include three or more components, as this is mostly a matter of using more wavelengths so that at least one equation can be obtained for each component in the system.

For best results with a 4 cm. absorption cell, the concentrations of chlorophyll and carotene are about 1.5 and 0.5 mg./l, respectively. The volume of solution actually used in the measurement need be only about 20 cc. and therefore the total amounts needed are only 0.03 mg. of chlorophyll or 0.01 mg. of carotene. This makes it possible to study the pigment content of very small amounts of plant tissue.

Any possible photochemical change is reduced to a minimum in the present form of the apparatus, for the absorption cells are placed behind slit 2 of the monochromator and the solution is thus exposed only to the very low intensity of the radiation actually used in the measurement (about 10^{-8} lumens) and not to the full intensity of the condensed beam of the total radiation of the source, as was the case in the earlier set-up⁽²⁾.

Errors of incomplete extraction of the pigment from leaf material are inherent in any method in which an extraction is made. Dilution errors can be made very small by the use of calibrated volumetric devices. To demonstrate that Beer's law is obeyed, it is advisable to make up a number of solutions of pigment mixtures at the proper total concentration for the wavelength employed. If the relative proportions vary over the entire composition range, say from 5 to 95% for each component of a two-component system, and the percentage composition is plotted against

* Reprinted from an article on the fluorescence of chlorophyll, *Protoplasma*, Vol. 22, No. 4, 513 (1935).

(6) Zscheile, F. Paul, Jr. *J. Phys. Chem.*, **38**, 95, 1934.

(7) Miller, Elmer S. *Plant Physiol.*, **9**, 681, 1934.

(8) Miller, Elmer S. *J. Am. Chem. Soc.*, **57**, 347, 1935.

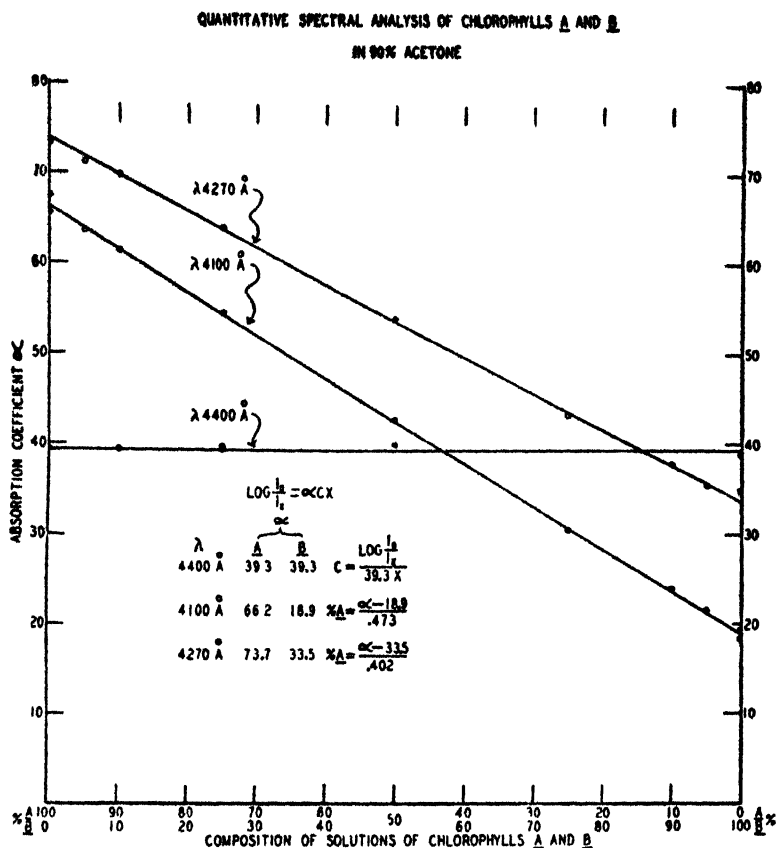


FIGURE III.*

SPECTRAL ANALYSIS OF CHLOROPHYLLS A AND B
IN 90% ACETONE

$$\text{LOG} \frac{I_0}{I_t} = \alpha C X$$

COMPOSITION IN TERMS OF A													
WAVE LENGTH	α		KNOWN A+B							UNKNOWN A+B		"C" FRACTION	
	A	B	5%	10%	25%	50%	75%	90%	95%	1	2	10 MONTHS	24 MONTHS
4100 Å.	66.2	18.9	5.50	10.5	24.3	50.3	75.3	89.9	94.2	65.3	63.5	56.3	25.2
4270 Å.	73.7	33.5	4.73	10.4	24.1	50.7	75.6	90.3	93.8	66.7	65.0	40.1	23.6
AVERAGE % COMPOSITION			5.11	10.45	24.2	50.5	75.45	90.1	94.0	66.0	64.25	48.2	24.4

TABLE III.*

the absorption coefficient, α , a straight line⁽⁶⁾ is obtained when Beer's law is obeyed in the concentration range employed. In cases where purification of one pure component is difficult, or if the wavelength employed must be on the steep portion of an absorption band, these α -composition curves are useful for a more accurate determination of α for the pure components.

* Reprinted from an article on chlorophyll *Botanical Gazette*, Vol. XCIV, No. 4, 529 (1934).

An accurate knowledge of the entire absorption spectra of the pigments is necessary to determine where the absorption curves cross one another. The wavelength at which this occurs may be used to analyze unknown binary mixtures for total pigment. It is unsound to choose a wavelength for this purpose at random because of convenience of light source, without careful consideration of the exact spectra of the components present in the system. Single lines from a line

source have been used by some workers for the determination of total chlorophyll in extracts. If comparison were made between the total chlorophyll contents of a number of plants submitted to different environmental conditions, the assumption must be made that the ratio of the chlorophyll components was constant for all of the plants and that it remained constant during the experiment. This assumption is too wide for high accuracy. If only two components were present, only by chance would their curves cross at the wavelength of any particular line isolated from such a source. Such experiments leave the problem of distinction between the components unsolved and may not furnish accurate analyses of total pigment.

In the use of this analytical method, accurate calibration, including determination of the spectral region isolated, becomes more important when the wavelengths employed are on steep portions of the absorption curves or when the maxima are very sharp. It is advantageous to use slits as narrow as possible at all times, for the absorption maxima will then more nearly approximate their theoretical sharpness. This may offer a wider range of wavelengths suitable for analytical purposes and will permit a wiser selection. However, analytical systems in which wide slits are used may operate for some substances, particularly those having broad absorption bands, and give quite accurate analytical results, but the absorption curves may differ from those obtained with narrow slits. Fortunately the carotenes in an 80% ethanol—20% ether mixture have comparatively broad bands in the blue region of the spectrum and the differences in magnitude permit the use of a considerable number of wavelengths for purposes of accurate analysis.

Although it has long been assumed that chlorophyll is the pigment directly involved in photosynthesis, the parts played by its various components and by the yellow pigments remain unknown. Whether one component is ever present without at least small amounts of the others, and what their relative efficiencies are in photosynthesis, are questions awaiting answer. Miller⁽⁸⁾ analyzed leaf samples of four sugar cane hybrids and found that the total carotenoid contents of two were about two-thirds those of the other two. By visual observation, these low-carotenoid hybrids contained less than one-half as much chlorophyll as the others. It is pertinent to the present discussion that the lighter-colored hybrids, with low chlorophyll content, were far more efficient in photosynthesis than the greener ones, as measured by yield of sugar per acre.

Much work is being done on the kinetics of photosynthesis, in attempts to elaborate a theory of the steps involved, in accordance with the best

experimental data available. Any system of kinetics must take into account at least the concentration of total chlorophyll, unless it can be shown that chlorophyll is not active. If the concentration, not only of total chlorophyll but of its various components and of the yellow pigments, could be determined on leaves or cultures whose photosynthesis was being studied, probably further insight could be obtained into the kinetics of the process.

Many biologically-occurring compounds, including the leaf pigments, are difficult to isolate in the pure state and, once prepared, it is difficult to prove that the preparation is identical with the naturally-occurring substance and has not undergone change during its purification. This analytical method, used as a critical tool of purity determination, is offered as a partial solution to this problem (partial because it is thus far adaptable to extracts rather than living tissues). After two supposedly pure pigments have been isolated, their absorption spectra are determined in an inert solvent convenient for quantitative extraction from the plant tissue, and an analytical scheme is developed for certain wavelengths. Extracts in this solvent containing unknown proportions of the two pigments are then analyzed spectroscopically, using the same wavelengths. If all of the wavelengths agree on the percentage composition of the extract, it is very good evidence that the pigments have undergone no change during the purification from the extract to the pure preparations, for the absorption spectrum is perhaps the physical property of a pigment most sensitive to chemical change. Possible decomposition occurring in the steps from the original tissue to the extract must be due to the solvent or to the physical mixing of the tissue parts. Such changes must be avoided as much as possible by proper choice of solvent and extraction technique.

Some evidence has been presented elsewhere⁽⁹⁾ that a chlorophyll component *c* exists and this type of spectro-photoelectric data so far presented confirms its existence (Table III). Consult (3) for details. A chlorophyll fraction containing component *a* as an impurity gives evidence of a third component (not *b*), which changes after a long period to component *b*. Its possible isolation is a problem for the future.

The specific roles of the separate components of chlorophyll have never been satisfactorily demonstrated, for we do not know why more than one component occurs. Are both active in photosynthesis and, if so, are they interchangeable in the photochemical steps or do they react separately at different stages of a chain reaction? What factors, environmental and genetic, influence the component ratio? Do any of the yellow pigments take part in photosynthesis directly or

can they interfere with its progress? Such questions as these arise immediately when the primary light reaction of photosynthesis is considered.

It is probable that the apparatus under discussion could be modified in such a way that *in vivo* experiments could be made upon leaves and algal cultures with highly monochromatized light. The tissues used in such experiments, although small in quantity, could be spectroscopically analyzed for the various pigments after the physiological experiment was complete. The sampling error would thus be eliminated or greatly reduced. Such experiments on living photosynthetic systems, influenced in their design by accurate photochemical knowledge of the pigments involved, would offer possibilities of a better understanding of the fundamental processes of photosynthesis.

The spectro-photoelectric method described here is not presented as a final solution to problems involved in photosynthesis but as a method well adapted to the further investigation of some of these problems. With this in view, the foregoing considerations of apparatus, methods, and analysis applied to photosynthesis is presented for your discussion.

DISCUSSION

Dr. McAlister: Have the photocell, the amplifying system, and the galvanometer been tested for linearity of response (either separately or together), i.e., has it been shown experimentally that the galvanometer deflection is directly proportional to intensity of radiation striking the photocell (for the usual range of deflections employed) with an accuracy of 1 part per 1000?

Dr. Zscheile: For deflections of 100 cm. or less, the response of the amplifying system and galvanometer to an impressed voltage is linear to 1 part per 100 or better. The linearity of response to light of the entire system, including photoelectric cell, amplifying system, and galvanometer was tested by means of black silk screens at several wavelengths in the visible and ultraviolet regions. The transmission values of the screens (measured with a thermopile) were checked within 1% by the photoelectric apparatus. The method of mounting the screens in the light path was not sufficiently precise for greater accuracy.

Dr. McAlister: Is there a "dark current" present in the photocell, and if so, is it a function of previous exposure of the cell to radiation?

Dr. Zscheile: In the cell we are using now, there is a small "dark current" of the order of 10^{-18} amperes. This is balanced out by adjustment of the amplifying circuit. The "dark resistance" of the cell is 10^{16} ohms, much higher than that of the ordinary type of cell. The "dark current" of

the photoelectric cell may be eliminated by grounded guard rings on the inside and outside surfaces of the cell. The photocell's response is not affected by previous radiation. This is shown by the immediate return of the galvanometer reading to zero at any point in the progress of measurements when the shutter is closed.

Dr. French: By placing the photocell behind the second slit, does fluorescent light from the chlorophyll enter the photocell and does that cause any appreciable error?

Dr. Zscheile: The photocell is about 10 inches from the absorption cell, so that only a very small fraction of the fluorescent radiation would reach the light sensitive surface. This would not cause an appreciable error.

Dr. Arnold: If in your method of analyzing for the components of chlorophyll, one were to use prominent emission lines in the red regions of the spectrum (lines from a neon discharge tube, for example) it should be possible for anyone to determine by means of a spectrophotometer the amounts of chlorophylls *a*, *b*, and *c* present in a given material without separating out the carotene and xanthophyll.

Dr. Zscheile: Yes, such an analytical system seems feasible. Its development would depend upon the nature of the absorption curves in the red region in the particular solvent used in relation to the spectral lines which could be isolated satisfactorily by a spectrophotometer.

Dr. Brackett: Were you able to eliminate the influence of the transmission of the monochromator on measurements of emitted radiation, in the case of fluorescence? The transmission of the monochromator varies from wavelength to wavelength and would modify the observed intensities.

Dr. Zscheile: In the fluorescence study, no correction was applied for the change of monochromator transmission with wavelength. This change was probably very small over the spectral range studied. A correction was applied for the change of photocell sensitivity with wavelength.

Dr. Brackett: The specification of the sensitivity of a photocell or of a photocell plus amplification system in terms of lumens leaves one rather much in the dark. As I remember, the sensitivity curve of caesium caesium-oxide on silver is high in the blue, low in the green, rises to a maximum in the red and falls to zero at about 11,000 Å. The value to which it rises in the ultraviolet depends to a large extent on the material and thickness of the window. To evaluate its sensitivity in terms of lumens requires weighting in terms of the visibility curve and a knowledge of the wavelength distribution of intensity of the source.

Dr. Zscheile: The intensity in lumens at the absorption cell of the photometer system was an average value and was stated to give only an approximate idea of the order of magnitude. The calibration lamp was a tungsten filament lamp, operating at 105 volts, with a filament temperature of about 1900° K. No filters were used.

Dr. Brackett: A determination of the current output compared with incident radiant power at each wavelength would be of great value in comparison of methods.

Dr. Knorr: Can you give the history of the chlorophyll *b* used in the study of the fluorescence spectra; how long had it been extracted, and how long had it been in solution?

Dr. Zscheile: The pigment was isolated from fresh barley leaves by a combination and extension of the methods employed by Willstätter and Stoll and by Tswett. Component *b* was finally separated from the last traces of *a* by fractional

precipitation from an ether petroleum-ether mixture. The preparations were 6 months old and the *b* component had been in ether solution 2 months. The preparation of *a* had been in ether solution 8 days. These solutions were kept in tightly stoppered bottles in the dark and had not lost their original colors. The apparatus was not in readiness for the fluorescence measurements when the chlorophyll preparations were made and scarcity of material prevented the use of fresher solutions.

Dr. Knorr: Was the phase test positive?

Dr. Zscheile: The preparations gave good phase tests.

Dr. Knorr: Was the chromatographic method of separation used in the preparation of the *b* component?

Dr. Zscheile: In the final separation from component *a*, the *b* was precipitated and filtered on talc, the talc absorbing the *a* and leaving *b* as a solid layer above the talc.

LIGHT INTENSITY AND CARBON DIOXIDE CONCENTRATION AS FACTORS IN PHOTOSYNTHESIS OF WHEAT

F. S. BRACKETT

The purpose of this paper is to show to some extent the possibilities and limitations which are to be found in the investigation of photosynthesis in higher plants and to offer certain observations regarding mechanism. The experiment upon which these conclusions are based was originally undertaken as a preliminary to work with monochromatic light in order to determine whether further work with higher plants, such as wheat, would be justified. As a result of an analytical treatment of the data obtained, one is led to the conclusion that such material lends itself to the attack on certain restricted problems. The experiment was conducted by Mr. Hoover working under my direction at the Smithsonian Institution in 1932. It will suffice for our purpose to point out simply the salient features of the experiment. For greater detail one may refer to the original article, Vol. 87, No. 16, of the Smithsonian Miscellaneous Collections.

Wheat plants, growing in nutrient solution of assigned concentration held at constant temperature, were mounted so that the leaf surfaces were confined in a glass inclosure. Air with a constant CO_2 concentration was supplied to the chamber at a fixed rate. The air was recirculated over the leaves at a high rate compared to the supply rate. CO_2 concentration was measured in both intake and exhaust by a method developed at the Fixed Nitrogen Laboratory by Ernest C. White. Essentially this method depends upon the measurement of conductivity of a potassium hydroxide cell wherein .02 N potassium hydroxide solution is brought into equilibrium with a fixed proportion of air at a definite controlled temperature. In order to make this method applicable it was necessary to improve the accuracy of thermostating wherein the temperature fluctuation was limited to $\pm .05^\circ\text{C}$. Polarization was eliminated by reversal of direction of current. This method proved to yield values of CO_2 concentration reproducible to less than 1% of normal air concentrations.

In the course of this experiment we obtained homogeneous data for CO_2 concentrations varying over a range of 100 times, for approximately 0.1 to 10 times normal air concentration. Light intensity was varied from 80 foot-candles to 1,000 foot-candles, that is, the maximum corresponded to approximately one-fourth gram calory per square centimeter per minute, or one-fourth of ordinary sunlight intensity.

The illumination was supplied from eight symmetrically arranged tungsten lamps, thus reduc-

ing self-shading to a minimum. Furthermore, since each leaf, no matter what its orientation, was subjected to practically equal illumination on both faces, the variation in radiation density was reduced to a minimum. Nevertheless, even such a measure fails to eliminate the difficulties arising from the variation of energy density within the leaf. It was found that the leaves transmitted from 14 to 18% of visible radiation. However, since most of this transmission occurred in the yellow and green, it is inevitable that total absorption took place in the region of the greatest chlorophyll absorption. While some transmitted energy is detectable even in the region of 6600 Å, this may be attributed to the absence of chlorophyll in certain portions of the leaf. Furthermore, with radiation arising from a high temperature tungsten lamp, the greatest energy is to be found at the long wavelength or red end of the spectrum, with a rapid decrease in energy as one goes toward the blue. Consequently, the contribution of absorbed energy will arise chiefly in the red end of the spectrum.

Radiation from the high intensity mazda lamps was filtered through a screen of copper sulphate solution, thus reducing the infrared radiation to a point where the ratio of visible to infrared approached that of sunlight illumination. Under those conditions normal coloration and growth were obtained. The copper sulphate solution serving as a radiation filter was circulated directly over the confining walls of the growth chamber itself. This was maintained by thermostating the circulating copper sulphate solution.

In these experiments, observations made over this wide range of CO_2 concentrations (0.1 to 10 times normal air) were obtained at six different representative light intensities. Growth corrections were made based upon the photosynthetic rate under standard conditions. Values for dark respiration were determined at intervals during the experiment, then assuming no change of respiration during illumination, values were arrived at by interpolation which were added to the observed rate of CO_2 assimilation.

Figure 1 shows the results of this experiment, the photosynthetic rate as measured by the amount of CO_2 absorbed in a given interval of time plotted as ordinates (V) and the CO_2 concentration as abscissae (S), proportionality factors being as follows:

Ordinates, CO_2 assimilated. Multiply by 0.00025 to obtain cc. per min.

Abscissae, CO_2 concentrations. Multiply by

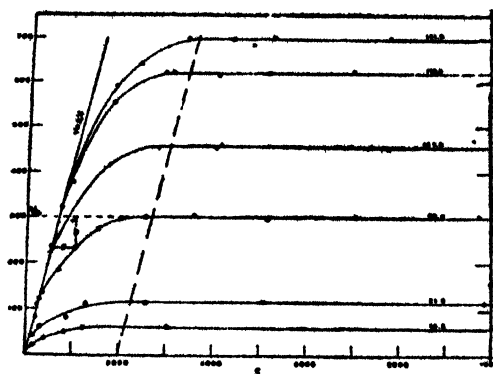


FIGURE 1

0.000041 to obtain volume per cent. The individual curves represent values obtained for six light intensities, the factors being as follows:

Parameters, light intensity. Multiply by 3.56×10^{-4} to obtain watts/cm². Multiply by 4.96 to obtain foot-candles.

Thus we have a fairly representative family of curves wherein the light intensity is the parameter. Of course these data may be replotted as photosynthetic rate against light intensity where CO₂ is the parameter, as shown in Figure 2. It will be noted that at high CO₂ concentra-

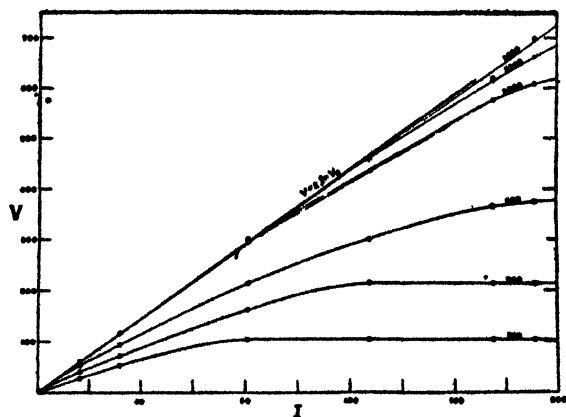


FIGURE 2

tions the photosynthetic rate is directly proportional to the light intensity.

Turning again to Figure 1, this point will be borne out by the fact that the photosynthetic rate is the same for all high values of CO₂ concentration for a given light intensity. Other than this, the most striking feature of the curves is the fact that the transition occurs for about the same range of CO₂ concentration for each curve. If one strikes a line through the points for lower CO₂ concentrations as indicated by $V = K_1'S$, it will be seen that this transition range is bound-

ed by another line parallel to the first approximately through the value of $S = 2,000$. In other words, for high light intensity, the photosynthetic rate rises at first almost linearly with CO₂ concentration, then passes through a transition range, finally becoming independent of CO₂ concentration. In an attempt to represent these various curves analytically, it seemed likely that a consideration of the distance over from the limiting line $V = k_1'S$, which I have indicated by a in Figure 1, and downward from the maximum, which I have indicated by b , might lead to a common representation. As a matter of fact, we are led to a consideration of the fractional part V_m/b against a . It is evident that the distance a may be expressed as $S - (V/k_1)$, which I shall call x . The ratio of V_m to b I shall call y , that is $y = V_m/(V_m - V)$. As a further refinement, however, it was found that the fractional values of the maximum were better described in terms of a line of greater slope, which I will call k' , than the limiting line indicated. The significance of this fact will be discussed later in the paper.

Plotting x against y , we find that the curve is readily represented by the expression

$$x = (\log_e y/c_1)/c_2.$$

In other words,

$$S = V/k' + (1/c_2) \log_e [V_m/c_1(V_m - V)].$$

Thus, we obtain the expression for V :

$$V = V_m [1 - e^{-c_2(S - V/k' + (1/c_2) \log_e c_1)}]$$

Plotting $\log_e [V_m/(V_m - V)]$ against $S - V/k'$, as shown in Figure 3, we find that all the points lie within the experimental error of a straight line whose slope c_2 equals $1/473$ and whose intercept value, $\log c_1$, is .25. In this plotting, we have found that the best value of k' is .57. Turning to our equation for V , we may regard the expression $(1/c_2) \log_e c_1$ as a correction to S , whose value is 117. Taking our values for the maximum and plotting them against I , Figure 4, we obtain an expression

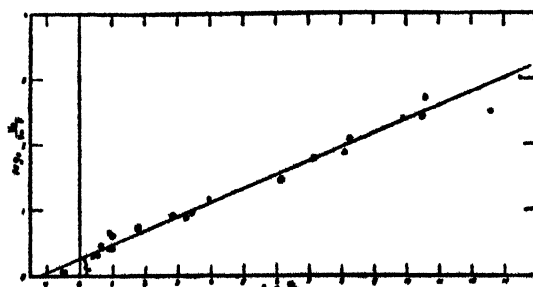


FIGURE 3

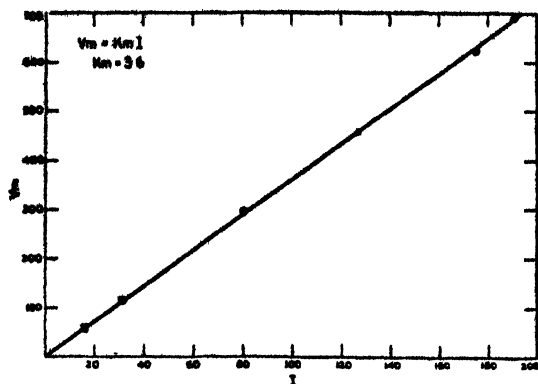


FIGURE 4

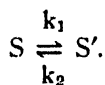
$V_m = k_m I$, where $k_m = 3.6$. This may now be introduced in our equation for V , giving

$$V = k_m I \left[1 - e^{-c_2 \left(S - \left(\frac{V}{k'} + \left(\frac{1}{c_2} \right) \log_e c_1 \right) \right)} \right]$$

This expression is simply for the moment an analytical representation of all the points observed (with the exception of those which arise in the limiting slope), wherein we have four arbitrarily determined constants k_m , k' , c_1 , and c_2 .

In order to arrive at the significance of this equation, let us consider the following mechanism:

First, diffusion, which we will represent by



Second, photochemical velocity:

$$V = c' I (1 - e^{-c'' S'})$$

The factor $1 - e^{-c'' S'}$ we recognize as closely analogous to the total absorbed energy according to Beer's law. In this case, however, it should be borne in mind that CO_2 is itself non-absorbing in the region of photosynthesis. Consequently the significance of this expression must be subjected to further analysis. This will be discussed later in the paper.

Finally, let us take into consideration a respiration whose velocity we shall represent by r . Then if we assume that equilibrium has been reached, the conservation of mass yields the following expression:

$$dS'/dt = k_1 S + r - k_2 S' - V = 0.$$

So we obtain for S'

$$S' = \left(\frac{k_1}{k_2} \right) (S) - \left(\frac{V}{k_1} \right) + \left(\frac{r}{k_1} \right)$$

and, substituting the equation for the velocity of the reaction, we have

$$V = c' I \left[1 - e^{-c'' \left(\frac{k_1}{k_2} \right) (S) - \left(\frac{V}{k_1} \right) + \left(\frac{r}{k_1} \right)} \right]$$

It will now be evident that we can identify c' of our theoretical equation with k_m , $c'' k_1/k_2$ with c_2 , k_1 with k' , and r/k_1 with $(1/c_2) \log_e c_1$. In other words, $(1/c_2) \log_e c_1$ may be identified as a respiration term.

For convenience we may prefer to think of r/k_1 as a correction to S ; in other words, due to respiration, an amount of carbon dioxide has been made available, r/k_1 , in addition to the external supply, S , the magnitude of this additive correction being 117.

Referring to the original data, we find that the value of r is of the same order of magnitude as the observed dark respiration value. On the basis of the four constants thus evaluated, we may plot a family of curves for the sake of comparison with the original data as shown in Figure 5. This

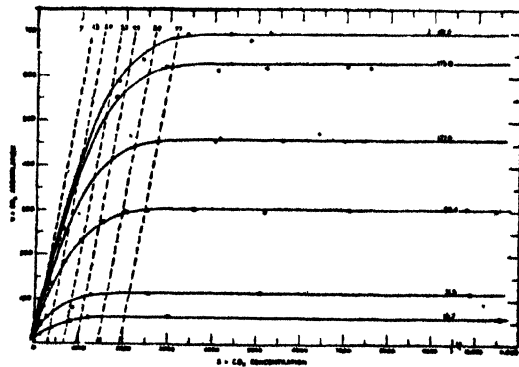


FIGURE 5

has been done conveniently by means of the $y = \text{constant}$ lines. On these lines every point represents an equal fractional part of the maximum value for that light intensity. We now see that the significance of our S -correction due to respiration may be seen in the fact that the curve extrapolates to a point on the S -axis 117 to the left of the origin. In other words, whereas the velocity was corrected for the dark respiration value, the corresponding contribution to the available CO_2 was neglected in the original plotting.

It will be seen that this analysis of the data radically differs from the previous explanations in that a factor $1 - e^{-c'' S'}$ has been suggested from the analysis of the data instead of an expression of the form arising from the simplest Langmuir adsorption isotherm. This latter type of consideration leads to an expression

$$a S' / (b + S')$$

If now, however, we take into consideration

the fact that a rapid decay in energy density takes place within the leaf and assume for the moment that the decay follows a more or less logarithmic form, we will arrive at the expression given analytically for the absorbed energy if we assume that the amount of energy finding its way into the photosynthetic reaction depends linearly upon the concentration of CO_2 or carbonic acid. If the adsorption of CO_2 or carbonic acid upon chlorophyll were to follow the simplest Langmuir isotherm, one would have

$$V = c' I (1 - e^{-c''(a'S/(b + S'))}).$$

Then if we assume that b is sufficiently large, i.e. large dissociation constant, the expression reduces to the one obtained from our analytical derivation.

It is evident, however, that unless the decay curve of energy density could be accurately known, such a conclusion in regard to the value of the dissociation constant could not be regarded as being based upon very substantial evidence. Certainly in this case we have no reason to suppose that the decay curve should necessarily follow Beer's law and only in such case would we be justified in arriving at a definite conclusion regarding the magnitude of the dissociation constant.

As an alternative to such considerations, we may regard the observed curve as the summation of a number of curves for light intensities grading from the maximum down to zero. It is immediately evident that the summation of a number of linear portions over a certain range leads to a resultant curve which is also linear over that range, though of less slope than the maximum; furthermore, that such a resultant will pass through the same origin as would the components. Pursuing this line of argument, it is evident that regardless of the exact form of the component curves they must extrapolate to the same origin as the observed curves. Consequently, we are justified in attaching some significance to the respiration value as a correction to the diffused CO_2 concentration as it makes itself felt in the transition range.

Turning again to Figure 1, I wish to point out that whereas the transition curves extrapolate to a point to the left of the origin, as explained by respiration considerations, the limiting values obtained for high light intensities definitely extrapolate to zero for CO_2 concentration. In other words, for higher CO_2 concentration values, the available concentration of diffused CO_2 or carbonic acid is influenced by the respired CO_2 , but for low CO_2 concentration values the respired CO_2 is not available. It may be significant that the effect of the respired CO_2 only makes itself

felt in the range where the photochemical mechanism begins to act as a limiting consideration, so that the rate never exceeds a value which is strictly proportional to the external CO_2 concentration. This is further brought out even at higher light intensities which were observed in the course of an earlier experiment.

It should be especially pointed out that while diffusion influences the shape of the transition curves in that it determines the slope of the $y = \text{constant}$ curves, diffusion limitation would be approached as a limiting slope only at very low CO_2 concentrations. Before this point is reached, however, some other CO_2 limitation asserts itself, as indicated by the $V = k_1' S$ line in Figure 1. These observations are in line with Warburg's contention that CO_2 enters in two different ways into the photosynthetic process.

In conclusion, I wish to point out: 1) that where adequate measures are taken to control the environmental conditions, higher plants yield highly reproducible values, that the experimental error is certainly as low as the best work with Warburg type of equipment on unicellular forms; 2) that by the use of a standard photosynthetic rate as a basis for correction for growth, homogeneous data may be obtained even over several days observation, which makes possible the representation of a whole family of curves by a simple type of analytical expression with a minimum number of significant constants; 3) that where radiant energy is supplied in a region of maximum absorption, total absorption takes place even with as thin leaves as wheat, so that the fall in intensity toward the center of the leaf must be considered even under conditions of cross-firing. Consequently, attempts to account for the shapes of the curves in terms of chemical kinetics without taking into account the intensity distribution will certainly lead to erroneous conclusions. Finally, we may point out that were monochromatic light used, of a wavelength corresponding to a maximal transmission for chlorophyll it may be possible to largely eliminate the effect of inhomogeneous energy density distribution within the leaf. In view of the high reproducibility and consistency of the data obtained, such investigations might well be expected to yield significant data regarding mechanism.

Furthermore, the method described lends itself to the study of the physiological effects produced genetically as exhibited in the albino and various pale green corn plants shown by Dr. Demerec. The pale green forms may especially lend themselves to a further reduction in the effects of inhomogeneous distribution of energy density within the leaf.

DISCUSSION

Mr. Lineweaver: Brackett suggested in his original report of this data that the carbon dioxide concentration might vary over a considerable range for different chloroplasts. One may employ this suggestion to derive an equation of the exact form of his final logarithmic equation. The following treatment was made after Brackett had shown me his equation, and when it was believed that the light intensity was practically the same for the different chloroplasts.

We may assume under ideal conditions that at constant light intensity the velocity of photosynthesis, $v = (k_1 P_{CO_2}) / (k_2 + P_{CO_2})$, is of the form of the simplest type of Langmuir isotherm. However, if the concentration of CO_2 is not the same for the different chloroplasts, as would result if diffusion through (not into) the plant were limiting, the following treatment may be desirable. We may divide the plant into a number of arbitrary cells, so that the concentration of CO_2 is constant in any given cell. The observed velocity, v_{obs} will then be equal to the summation of $v_1, v_2, v_3 \dots v_n$;

$$v_1 = (k_1 S_1) / (k_2 + S_1) , \\ v_2 = (k_1 S_2) / (k_2 + S_2) , \text{ etc.}$$

The observed velocity may also be expressed as $v_{obs} = (nk_1 S'') / (k_2 + S'')$, where n is the number of cells and S'' is the concentration of CO_2 which if uniformly distributed would give the observed velocity. It is now necessary to define S'' as a function of P_{CO_2} . It is easily seen that at low P_{CO_2} the value of S'' required would be considerably less than the concentration in the first cell and that at high P_{CO_2} when saturation is progressively reached in cells 1, 2, 3, etc., S'' will approach the value in cell 1 so that the curve $S'' \times S$ (or $S - v/k'$ if there is a boundary diffusion resistance) will be concave upward. The function $S'' = k_2 (e^{(S-v/k')} - 1)$ will qualitatively give such a curve. Inserting this value of S'' into the former equation one obtains

$$v_{obs} = nk (1 - e^{-(S-v/k')})$$

which is in the form of the final logarithmic equation.

The postulate given here is admittedly arbitrary, but at least gives an analytically feasible interpretation of the equation, assuming constant light intensity. Furthermore, it rests essentially on the hyperbolic nature of the light and carbon dioxide functions which have been employed mechanistically to give correct qualitative prediction of temperature coefficients, of the effect of inhibitors and of the results of flashing light ex-

periments (Burk and Lineweaver, *Nature* 135, 621 (1935)).

Dr. Brackett: I have been concerned about the contribution of diffusion in case there were a different diffusion resistance to different chloroplasts. Having come to the conclusion, however, that different intensities exist at different chloroplasts, it seemed that this would be sufficient to account for the observed curves, if one assumed a constant diffusion resistance. On the basis of the principle of simplicity, I was not inclined to look further for the explanation, particularly in view of the likelihood that a higher plant is so constructed as to provide a minimum diffusion resistance to the various chloroplasts.

Dr. Burk: I am inclined to believe, from considerations of any data at hand, that rather contrary to the opinion of Brackett, there would be something like equal orders of probability in regard to light intensities varying throughout the plant, as suggested by Lineweaver. The principle of simplicity, known for ages as the principle of William of Occam, forbids the assumption of unnecessary hypotheses, but eliminates no reasonable, untested explanation.

Dr. Brackett: One point might be mentioned if we go back to the consideration of the shape of the curves for CO_2 assimilation as a function of CO_2 concentration at constant light intensity. Over a rather wide range of CO_2 concentration, one finds practically constant assimilation rate. In order to account for this on the basis of the simplest Langmuir isotherm one would have to postulate a much lower dissociation constant than would be found if one attempts to fit the transition range directly. However, in view of the lack of homogeneity of energy density the observed curve may be regarded as the sum of a number of curves of this Langmuir type with a relatively low dissociation constant which is compatible with the constancy of assimilation for the higher CO_2 concentrations. This would bring our observations into harmony with the assumption of the simplest Langmuir isotherm to be discussed at greater length by Burk.

Dr. Arnold: Did you make any experiments with higher light intensities?

Dr. Brackett: Yes, we worked at higher light intensities in a preliminary experiment, which, however, was subject to certain objections regarding the method of growth correction and other matters of technique. For instance rubber connections were used, which were later replaced by an all vitreous system. Due to diffusion through the rubber connections, errors were introduced at low CO_2 concentration. In spite of these objections, the experiment offers certain points of interest. If one plots rate of assimilation

against light intensity at constant CO_2 concentration, the curve for high CO_2 concentration presents for lower light intensities a wide range of practical linearity. Then at approximately 1000 foot candles it breaks, increasing thereafter almost linearly, but at a much reduced slope. When the CO_2 concentration is still greater one obtains the same constant for the lower portion of the curve. For the range above 1000 foot candles the slope is increased slightly, but still far below the slope indicated in the lower range. Thus for light intensities above 1000 foot candles, one finds that the photosynthetic rate is dependent upon both CO_2 and light intensity. The values obtained in this region cannot be obtained from the relationships which we have derived in the lower range. Evidently for this range of high light intensity and high CO_2 , we are dealing with a definitely different situation from that which we have reported in some detail in the case of our second experiment, where all of the values of light intensity were below 1000 foot candles.

Dr. Emerson: Your curves for rate of assimilation as a function of carbon dioxide concentration suggest that at the higher light intensities, diffusion of carbon dioxide through the stomata may have entered as a rate-determining factor.

Dr. Brackett: Yes, that is a possibility, and also there is the possibility of reorientation of the chloroplasts or chromatophores. If this were the case, the reduction in effective absorbing area would account for a new and possibly changing factor of light dependence. To me the rather remarkable thing is that the effective absorption is apparently constant up to 1000 foot candles. Both the possibility you mention, readjustment of the stomata, and also the possibility of a readjustment of the chloroplast in higher light intensity may enter into the modified conditions observed at the higher range.

Dr. Emerson: I should think the cross-lighting used during your experiments would minimize the effect of changes in position of the chloroplasts.

Dr. Mestre: In certain plants, at least, under intense illumination the chloroplasts tend to collect into more or less dense aggregates which decrease the effectiveness of the absorption. I don't know whether this occurs in wheat or not.

Dr. Emerson: Have you any information about the behavior of the stomata of the wheat plant in intense light and at high concentrations of carbon dioxide?

Dr. Brackett: No, I haven't.

Dr. Emerson: Have you any experiments which would help to show whether the rate-limiting process at low concentrations of carbon diox-

ide is a chemical process or a diffusion process?

Dr. Brackett: No. Decisive evidence, such as would be obtained from experiments at varying temperatures, is not available. However, the diffusion constant which we have assumed in our analysis is not limiting even at low CO_2 .

The difficulty with the temperature experiments arises from the fact that it took almost a week to obtain data at one temperature, and the danger is that if one attempted to obtain a complete set of data with other variables such as temperatures one would be in trouble finally in that the plant had varied too much. One would have to restrict the modification of other variables if one were to follow the temperature change over a considerable range. This certainly could and should be done.

Dr. Burk: I wonder if it is a consideration against your proposed idea of the summation of component curves, that after all the velocity over that range of high CO_2 pressure is directly proportional to the light intensity—and that the summations give, curiously enough, the required linearity. You have obtained an extraordinarily good fit for your data, and some rather peculiar explanation is apparently called for.

Dr. Brackett: It would appear that to retain the linearity with light intensity would place a special requirement on the type of summation.

Dr. Burk: It is to be pointed out that the respiration correction applied to the carbon dioxide concentration abscissa appears, by Brackett's analysis, to effect the curves only at high carbon dioxide concentration. The simplest, *a priori*, view would lead one to believe that one would observe the largest effect at low carbon dioxide concentrations, where the plant suffers most from carbon dioxide limitation. There are other conceptions, however, whereby the converse expectation might hold.

Dr. Emerson: The fact that these curves descend to the origin might be interpreted as evidence that the respiration correction is nearly right. If respiration in the light were seriously different from the value measured in the dark, then one would not expect these curves to go so neatly toward the origin.

Dr. Brackett: Yes. The amount of respiration correction is relatively large at low light intensities. More than that, unless one complicates the expressions, one has to assume constant respiration independent of light intensity if we appeal again to the principle of simplicity. I am inclined to conclude from that, that respiration was constant throughout the experiment. This is not conclusive evidence but simply in the direction of simplicity.

The intercept value of 117 corresponds to a dark respiration of 67 which is a little over 3

times larger than the dark respiration value observed. However, I do not regard the method of determination by extrapolation as sufficiently accurate to yield a significant magnitude. It may be simply significant as to order of magnitude.

Dr. Emerson: How many times greater than the respiration is the photosynthesis of the wheat plant?

Dr. Brackett: The maximum value observed for assimilation was 700, with a dark respiration of 20. This would correspond to a ratio of 35, which is a fairly high value, and the limit has by no means been approached.

Dr. Burk: This ratio is about as high as ever obtained and several fold higher than obtained with most plants studied, where the ratio is usually 2 to 10. It is interesting to recall that in some of Warburg's most important experiments he was willing to work with a ratio of less than unity. In Brackett's experiments there were a negligible number of points where the ratio was as low as the order of unity.

The Smithsonian data probably represents the best family of CO_2 -I curves ever obtained. Harder obtained four CO_2 -I curves each with four points. These data represent six light intensity parameter curves with ten or more points over a much wider range with respect to CO_2 . We have the two explanations of variable light intensity (Brackett) and variable diffusion resistance through the plant (Lineweaver), which possibilities may well occur. I am strongly inclined to believe that an explanation based on a constant cell boundary diffusion (as in Brackett's analysis) together with a simple Langmuir CO_2 isotherm (cf. Lineweaver and Burk, J. Am. Chem. Soc., 56, 658, 1934, case VII) probably provides the most satisfactory mechanism, ex-

perimental and random errors being considered. This view takes much support from the fact that the dissociation constant for the assigned CO_2 function is of the same order as is obtained in most plants so far studied. (Burk and Lineweaver, this volume), and the fact that such an excellently linear curve is obtained with respect to light intensity at high CO_2 concentration.

Dr. Brackett: I think this may be said, that the analytical data are so definite and consistent throughout the entire set of curves that one may later, through further study, arrive at some evaluation of the light distribution and diffusion characteristics which may make it possible to use the material as a more stringent test for the proposed kinetics, but that is rather an ambitious experimental undertaking. However, the characteristics involved are common to other plants of the same variety. In other words, the characteristics we are considering are general characteristics, rather than things peculiar to the particular plant, such as, for instance, the concentration of chlorophyll and the condition of the plant and things of that sort. One would probably encounter greater difficulty in determining diffusion characteristics than light characteristics, which might quite well be determined.

Dr. Emerson: In using these curves as a basis of kinetic studies on photosynthesis, it seems to me it would be necessary to be quite sure that their shapes are determined throughout by the process of assimilation itself, and are not influenced by such factors as stomatal aperture.

Dr. Brackett: The only inherent evidence of such change comes at higher light intensities such as were observed in the first experiment but not in the second experiment which is presented in this analysis.

KINETICS OF PHOTOSYNTHESIS IN CHLORELLA

WILLIAM ARNOLD

Warburg (1926) showed that the process of photosynthesis in *Chlorella* could be considered to consist of two major reactions, one photochemical, the other (known as the dark or Blackman reaction) not involving light, but sensitive to temperature. This paper attempts to determine the extent to which the concept of a two-step cycle may be used to describe photosynthesis when effected by intermittent light, (Emerson and Arnold, 1932). From a general expression of the two-step cycle equations are derived to fit the experimental data for *Chlorella*.

Model of Photosynthesis: First Postulate

Photosynthesis is accomplished by a cyclic mechanism in which only two reactions are of importance in determining the rate. One is a photochemical change:



in which the rate is represented by

$$\phi_1(I, N)$$

where I is the intensity of the light and N is the amount of a present.

The other reaction, known as the Blackman reaction, is a temperature-sensitive chemical reaction:



with the rate given by

$$\phi_2(B, n),$$

where n is the amount of b present, and B is a function of the temperature.

Model of Photosynthesis: Second Postulate

If measured in the same units, the amount of a plus the amount of b is a constant.

$$N + n = K,$$

where K is a constant whose nature and measurement will be considered later.

Model of Photosynthesis: Third Postulate

The completion of the cycle is necessary for the liberation of oxygen.

The Steady-State of Photosynthesis in Continuous Light

In continuous light the rate of oxygen production Q will be

$$Q = \phi_2(B, n') \quad \text{I}$$

where n' (the steady-state value of n) is determined from the simultaneous solution of

$$\phi_1(I, N') = \phi_2(B, n'), \quad \text{II}$$

and

$$N' + n' = K. \quad \text{III}$$

Steady-State of Photosynthesis in Flashing Light

Throughout this section and those following devoted to flashing light, we assume that the Blackman reaction does not take place during the time that the light is on. This assumption is based on the fact that the light is on for only about a tenth of one per cent of the dark time.

The photosynthesis accomplished in flashing light may be most significantly expressed in terms of the amount of oxygen produced per flash; we shall call the quantity M . Experimentally M represents the amount of oxygen produced in five minutes, divided by the number of flashes in five minutes.

After the flashing light has been on for five minutes the transient terms will have become negligible; a steady-state will have been reached in that the cycle will exactly repeat itself. Let

$N_1 + n_1 = K$ (at the beginning of the flash) and

$$N_2 + n_2 = K \text{ (at the end of the flash).}$$

During the time of one flash the amount of substance a falls from N_1 to N_2 . These two numbers define a fraction G , such that

$$N_2 = GN_1$$

(The value of G depends on the order of the reaction and will not in general be independent of N_1 .) Similarly, the two values of n determine a fraction H :

$$n_1 = Hn_2.$$

Or, H is the fraction of b which lasts until the beginning of the next flash.

From the third postulate, that oxygen is produced only on the completion of the cycle, we see that

$$M = N_1 - N_2.$$

Now, since N_2 equals GN_1 ,

$$M = (1 - G)N_1, \quad \text{IV}$$

and the value of N_1 may be determined from the four simultaneous equations:

$$N_1 + n_1 = K \quad (A)$$

$$N_2 + n_2 = K \quad (B)$$

$$N_2 = GN_1 \quad (C)$$

$$n_1 = Hn_2 \quad (D)$$

Substituting in (B) the value of N_2 and n_2 given by (C) and (D) we have

$$GN_1 + n_1/H = K,$$

or

$$n_1 = H(K - GN_1).$$

Then, replacing n_1 in (A),

$$N_1 + HK - GHN_1 = K,$$

or

$$N_1 = K[(1-H)/(1-GH)]$$

Substituting the above value of N_1 in Equation IV:

$$M = K[(1-G)(1-H)/(1-GH)] \quad V$$

or the oxygen produced per flash

If the time between flashes is sufficiently long, the Blackman reaction can reduce the amount of b to almost zero; thus H becomes zero and

$$M = K(1-G). \quad VI$$

If the energy in one flash is large enough, all of substance a will be used up, G becoming zero. Then,

$$M = K(1-H). \quad VII$$

Therefore, if both the energy and the time between flashes are large,

$$M = K. \quad VIII$$

This is the experimental method of measuring K .

Application of the Model to *Chlorella*

In order to find expressions for ϕ_1 , ϕ_2 , G , and H , involving experimental conditions, we analyze the experimental results in *Chlorella* in terms of the model.

First, we fulfill by experiment the conditions implied in Equation VI. (Emerson and Arnold, 1932)

Secondly, we rewrite Equation VI to read

$$G = (K - M)/K.$$

If we plot the experimental quantity $\ln(K - M)/K$ as a function of E , the energy per flash, using the saturation value of M as K (Equation VIII), we see that it is of exponential form (Figure 1). Therefore,

$$G = e^{-AE}.$$

From the definition of $\phi_1(I, N)$ as the rate of the photochemical process, and from our as-

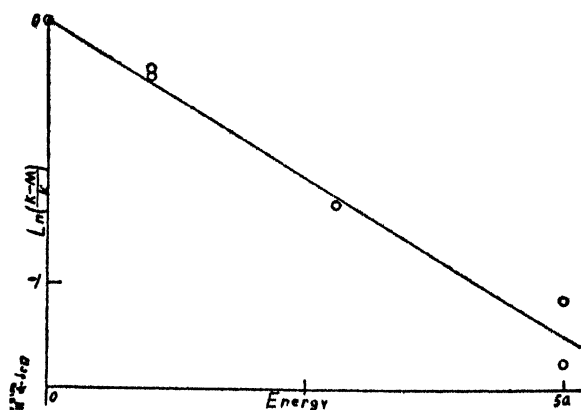


FIGURE 1

$\ln G$ as a function of the energy per flash. The straight line indicates that the light reaction is first order. (Data from Emerson and Arnold, 1932).

sumption that the Blackman reaction may be neglected during the short time that the light is on, we have

$$\int_0^w \phi_1(I, N) dt = N_1 (1 - e^{-AE}) =$$

$$N_1 (1 - e^{-A \int_0^w I dt})$$

where w is the length of the light flash and E is equal to

$$\int_0^w I dt.$$

Differentiating with respect to w (considered as a variable),

$$d/dw \int_0^w \phi_1(I, N) dt = [\phi_1(I, N)]_w =$$

$$N_1 A I_w e^{-A \int_0^w I dt}$$

the rate of the photochemical reaction at time w .

$$\text{Remembering that } N_1 e^{-A \int_0^w I dt}$$

is the value of N at time w ,

$$\phi_1(I, N) = AIN,$$

the rate at any time.

The determination of H and $\phi_2(B, n)$ is made

in the same manner. When the light flashes are very bright Equation VII is applicable, and

$$H = (K - M)/K.$$

In Figure 2 we have plotted $\ln[(K - M)/K]$ against the dark time d , (the length of time between flashes). The straight line indicates that

$$H = e^{-Bd}.$$

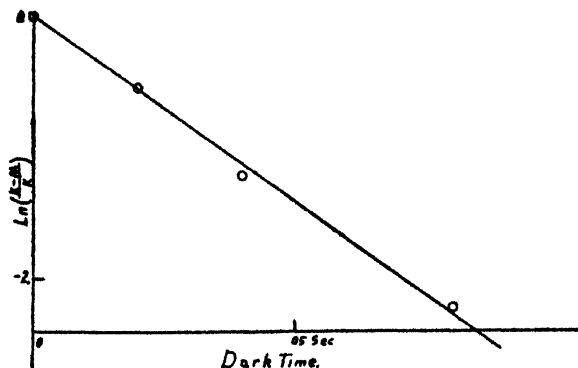


FIGURE 2

$\ln H$ as a function of the time between flashes. The straight line indicates that the Blackman reaction is first order. The slope of the line is B and depends upon the temperature.

From the definition of the quantities involved we know that

$$\int_0^d \phi_2(B, n) dt = n_2 - n_1 = n_2(1 - e^{-Bd}).$$

Differentiating both sides with respect to d we have

$$d/dd \int_0^d \phi_2(B, n) dt = [\phi_2(B, n)]_d = n_2 B e^{-Bd}.$$

Since, $n_2 e^{-Bd}$ is the value of n at the time d ,

$$\phi_2(B, n) = Bn$$

at any time.

The above equations, showing that the Blackman reaction can be represented as first order in terms of the intermediary substance b , may also be derived from a study of the rates of photosynthesis after irradiation with ultraviolet light. (Arnold, 1933).

We are now in a position to give explicit relationships for the rate of photosynthesis. Substituting in Equation II the expressions for ϕ_1 and ϕ_2 which we have just determined, we obtain

$$AIN = Bn, \quad IX$$

which, taken in conjunction with the conservation equation,

$$N + n = K, \quad X$$

can be solved for n . Substituting in Equation IX the value of N from Equation X,

$$AI(K - n) = Bn$$

or

$$n = (AIK)/(AI + B).$$

Putting the value of n in Equation I, we have

$$Q = K [(AI B)/(AI + B)] \quad XI$$

as the general expression for the rate of photosynthesis in continuous light.

The amount of photosynthesis per flash in flashing light is given (after substituting for G and H in Equation V) by the expression,

$$M = K [(1 - e^{-AE}) (1 - e^{-Bd}) / (1 - e^{-AE} e^{-Bd})] \quad XII$$

We have already seen that this equation fits the data when either E or d are very large. When that is not true, the denominator in Equation V will be less than 1, so that the value of M for small dark times will be raised in comparison with Equation VII. In Figure 3, the bottom curve was made with a neutral filter covering the discharge tube under conditions that made the value e^{-AE} equal to about .55. The change in shape due to the change in the denominator may plainly be seen.

The effect of small concentrations of indifferent narcotics on the process of photosynthesis is described qualitatively for both continuous and flashing light by assuming that the presence of a narcotic reduces the amount of material involved in the cycle, that is, reduces the value of K in the above equations.

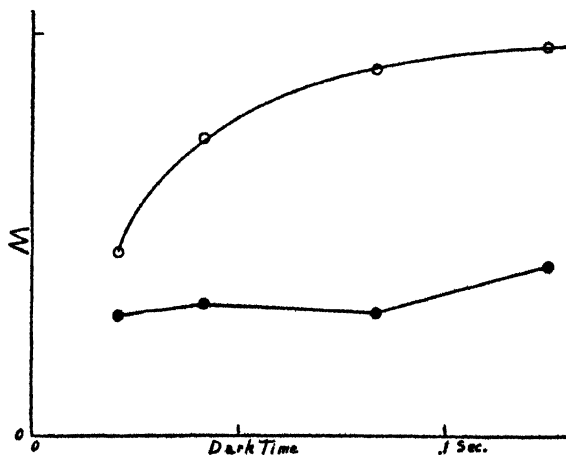


FIGURE 3

Relative photosynthesis per flash as a function of the time between flashes. Open circles were made at light saturation. Solid circles were made with a 25% neutral filter.

Small concentrations of HCN, on the other hand, have no effect on the amount of material involved in the cycle, but by diminishing the value of B make it necessary to allow a longer time for the completion of the Blackman reaction. That only B is affected by HCN is consistent with the observation that HCN does not inhibit photosynthesis at low light intensities where the rate would be given by

$$Q = AIK \quad \text{XIII}$$

That the value of K in the above equations depends upon the carbon dioxide concentration is clear from the fact that with intermittent light it is impossible to compensate for the effect of low carbon dioxide concentration by any spacing of the flashes. (Emerson and Arnold, 1932) For high-intensity continuous light, Lineweaver and Burk (1934) have shown that the reciprocal of the rate of photosynthesis bears a linear relationship to the reciprocal of the carbon dioxide concentration. Remembering that for high light intensity, Equation XI becomes

$$Q = KB \quad \text{XIV}$$

and that B does not depend upon the carbon dioxide concentration, we have

$$K = [U/(k_1 + U)]K_0 \quad \text{XV}$$

as the relation between K and U (the carbon dioxide concentration). k_1 is a constant and K_0 is the value of K at carbon dioxide saturation.

For the flashing light data we find empirically that K can be expressed by

$$K = [k_2 + (k_4 U)/(k_3 + U)]K_0 \quad \text{XVI}$$

when

$$k_4 + k_2 = 1.$$

The small constant k_2 may be interpreted as that part of photosynthesis which uses directly the carbon dioxide produced by respiration.

K_0 , the value of K at carbon dioxide saturation, has been found to be directly proportional to the chlorophyll content of the cells. (Emerson and Arnold, 1932). If K_0 be expressed in mols of oxygen produced per cmm. of cell material, and if the chlorophyll content be expressed in mols of chlorophyll per cmm. of cell material, we have the relation

$$K_0 = \text{chlorophyll}/\rho$$

where ρ has a value of 2000 — 3000. Exactly what this relationship means we do not know.

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DISCUSSION

Dr. Brackett: One would like to know the intensity distribution within a flash. With little inductance, the current might have died out logarithmically. If such had been the case, the larger part of the period of illumination would have been at low intensity.

Dr. Arnold: That is an important point, but we have not had the equipment necessary to study the intensity distribution.

Mr. Lineweaver: Do you think that there are any reasonable methods of avoiding the implication that since 2000 chlorophyll molecules are employed per CO_2 molecule reduced in flashing light experiments, the same number would be required in continuous light experiments?

Dr. Arnold: No, I don't think there are.

Dr. Burk: Isn't it fair to say that there is as yet no reasonable explanation of this 2000 number?

Dr. Arnold: Yes.

Dr. Rothmund: What would be the limit of error in the determination of the photosynthetic unit of 2000 chlorophyll molecules?

Dr. Arnold: The largest error is in the chlorophyll determination. (See Dr. Zscheile's paper). I believe the lowest value is the most significant.

Dr. Burk: Would the number be higher with low light intensity?

Dr. Arnold: Yes.

Dr. van Niel: If the assumptions as laid down in equations 1 and 2 be correct, then one has to admit the reality of these large chlorophyll units. But I should like to ask whether it is probable that the outcome of the calculations points to a fundamental discrepancy between these assumptions and the actual mechanism of photosynthesis.

Dr. Arnold: In my opinion, we either have to believe that a large number of chlorophyll molecules cooperate in the mechanism of photosynthesis, or we have to believe that some fundamental assumption which we have been taking for granted is wrong.

THE EFFECT OF INTENSE LIGHT ON THE ASSIMILATORY MECHANISM OF GREEN PLANTS, AND ITS BEARING ON THE CARBON DIOXIDE FACTOR.

ROBERT EMERSON

Injury to the photosynthetic mechanism of green plants as a result of exposure to intense light has been reported by both early and recent investigators of carbon dioxide assimilation. A diversity of experiments with technique of usually unproven reliability has led to opinions about the nature of the injury which are by no means in agreement. This lack of agreement becomes important because deductions concerning the mechanism of carbon dioxide assimilation have been made from the observed behavior of plants exposed to intense light. It seems to the writer that agreement on the descriptive characteristics of the process must precede any fruitful discussion of the mechanism involved. Some relatively simple experiments with the alga *Chlorella pyrenoidosa* may facilitate a more satisfactory general interpretation of the conflicting observations on the variability of the rate of photosynthesis during prolonged exposure to intense light.

Without attempting to discuss all the published work bearing on this subject, we shall consider representatives of three kinds of experiments which have contributed to the present state of our knowledge of this subject.

(1.) Sunlight has been concentrated with a burning glass on plant material, and the effects on cell structure, color, and behavior observed. Pringsheim (1879-1881) and Ewart (1898) concluded from such experiments that the chlorophyll could be partially or completely destroyed in a few minutes. As Ewart was aware, both his own results and those of Pringsheim probably were due in part at least to heat effects, and not to visible light alone, although Pringsheim believed that heat was not a factor in his experiments. Judging from Pringsheim's description of effects in different regions of the spectrum, he seems to have had good grounds for this opinion, but his precautions would be considered crude today. Ewart made qualitative observations on photosynthesis, and noted that it might be appreciably depressed without apparent change in green color, though he admitted that considerable changes in chlorophyll content could escape detection. In direct disagreement with Pringsheim, he concluded that "light acts both as a stimulus to the formation of chlorophyll and induces its photochemical oxidation". (p. 395). Pringsheim had reported that even after weeks the chlorophyll was not regenerated in bleached areas, though the cells involved continued to live.

(2.) Some investigators have observed the formation and disappearance of starch in leaves

exposed to strong light. Ursprung (1917) borrowed from photography the term "solarisation" to describe the phenomena which he and others observed with this method. On exposure to light, a leaf previously kept in darkness until free of starch will form starch, and presently will give a strong starch-iodine reaction. If the light is intense and the exposure continues for several hours, the starch gradually disappears, until finally the iodine test shows no blackening, or a reversal of the original effect of light. By projecting the spectrum of concentrated sunlight on a leaf, Ursprung showed that solarization set in first in the red region, later progressing toward the blue end. He does not compare the effect in green, where chlorophyll does not absorb appreciably, with the effect in red and blue, where absorption is strong. He says that light intensities sufficient to cause disappearance of starch may cause no apparent change in greenness, though extreme intensities cause destruction of chlorophyll and death of the cells. He believes that the disappearance of starch indicates cessation of carbon dioxide assimilation. This is very likely true, though certainly other factors besides photosynthesis govern the formation and disappearance of starch. Ursprung discusses several possible causes for cessation of photosynthesis in strong light, but his results do not permit a decision among them.

Holman (1930), using technique superior to Ursprung's, obtained similar results with intensities of incandescent light running up to about 150,000 meter candles. He made experiments to test some of the reasons proposed for the cessation of starch formation in strong light. The shadow of a 19- μ wire laid across an illuminated area was sufficient to permit starch formation, though solarization was complete on both sides of the wire. Holman concluded from this that heating and water-loss could be neglected as possible causes of solarization. Intensely illuminated areas regularly showed a loss of greenness, and he suggested that chlorophyll destruction may have been partly responsible for the disappearance of starch. Since no analyses of chlorophyll content were made, it remains possible that the changes in greenness were due to changes in position or degree of expansion of the plastids. Plastids are known to contract and move to less exposed positions in strong light (Senn, 1908).

Without good evidence, Ursprung and others have suggested that the accumulation of products of photosynthesis (oxygen, starch, or sugars)

might bring on solarization. Holman argued that if this were true an increase in carbon dioxide concentration, by increasing the rate of photosynthesis at the beginning of exposure, should hasten solarization. Consequently he raised the carbon dioxide concentration from 0.015% to 0.6% in air. Far from hastening solarization, this greatly delayed it, or prevented its appearance altogether. Unfortunately he made no attempts to show whether or not the 0.6% carbon dioxide caused an increase in photosynthesis, though in all probability it did so. Holman published no measurements of rate of photosynthesis in connection with starch formation and was more conservative than Ursprung in relating this process to photosynthesis.

(3.) The third group of experiments relating to this subject are those in which actual rates of photosynthesis were measured in different intensities of light. It is necessary to consider these experiments in more detail, because it will be shown that technique may play a large part in producing misleading results. It will be helpful to discuss experiments on submerged water plants and on the leaves of terrestrial plants separately, because in the latter case gas exchange is regulated by diffusion through the variable stomata, and in the former case it takes place freely through the plant or cell surfaces. In both cases, experiments may be cited where no injury has been observed at high light intensities, and others where the rate of photosynthesis has been observed to fall during exposure to strong light. The writer will attempt to show that the constancy or inconstancy of the rate may reasonably be attributed to the adequacy or inadequacy of the carbon dioxide provision, and then the tests of this hypothesis by experiments on *Chlorella* will be described.

A decrease in rate of assimilation by leaves exposed to intense light was reported by Willstätter and Stoll (1918). Analyses before and after exposure showed that there was no measurable decrease in chlorophyll content, even after prolonged maximal photosynthetic activity e.g., cherry laurel leaves exposed for 22 hours). They attributed the observed decrease in rate solely to accumulation of products of photosynthesis, without considering the possibility of a carbon dioxide shortage due to partial closure of the stomata. Not much is known about the behavior of stomata after long exposure to intense light. The experimental conditions used by Willstätter and Stoll were calculated to reduce the possible influence of stomatal aperture to a minimum, but the effectiveness of their measures was not tested, and Stålfelt has shown that at least in some plants, the stomatal aperture is subject to continual change (1929). Johansson (1929) and Johans-

son and Stålfelt (1928) have shown that in ordinary air the rate of photosynthesis is subject to relatively small changes in stomatal aperture. Willstätter and Stoll used 5% carbon dioxide, a concentration much higher than that in air, and small changes in stomatal aperture might be expected to be less significant. Johansson's conclusion was that under constant conditions in ordinary air, fluctuations in rate of assimilation were directly traceable to variations in stomatal aperture, and it is not unreasonable to suppose that in spite of the high carbon dioxide concentration used by Willstätter and Stoll the stomata may still have played a part.

Montfort and Neydel (1928) sought to show that fluctuations similar to those found by Johansson appeared also in the fronds of the fern *Trichomanes*, which does not have stomata. Their experiments were carried out under water, and as Johansson has pointed out, their rather primitive technique does not seem to have established the adequacy of their carbon dioxide supply.

Many investigators have tried to avoid the disturbing effects of stomata by experimenting on aquatic algae or submerged water plants. Both Harder (1930) and Arnold (1931) have found a declining rate of photosynthesis under supposedly constant external conditions, when their material was exposed to strong light. Harder measured rates of assimilation by Winkler oxygen titrations, Arnold by the bubble counting method supplemented by micro gas analysis. During exposure to light, the material was at rest in the suspending fluid, which was not constantly circulated. Harder says that in his experiments the fluid was mixed from time to time, but he records no experiments to show that his procedure provided the assimilating cells with carbon dioxide at a rate unlimited by diffusion, and considering the low initial concentration, the risk of limitation by diffusion is great, especially at higher light intensities.

Arnold explains that uncirculated water was essential in his bubble counting experiments, in order to establish the necessary "oxygen mantle". He believes that the carbon dioxide supply was adequate because he used bicarbonate solutions. This is an improvement over Harder's technique, but again no experiments are recorded to show that the procedure was adequate. Arnold tries to justify it by citing the work of James (1928), who found that rates of assimilation in standing bicarbonate solutions were equal to rates obtained in flowing solutions containing only dissolved carbon dioxide. Due to peculiarities of his technique James could not run his bicarbonate experiments longer than a few minutes, and they are consequently not comparable with Arnold's, which lasted for hours. Romell (1926), in an

excellent discussion of the availability of carbon dioxide under various conditions, shows that although a plant in bicarbonate is better provided with carbon dioxide than if the only source of supply is dissolved gas, nevertheless after the first few moments the rate of assimilation in standing fluid may become limited by diffusion even in highly carbonated waters. From the character of James' results, it seems evident that the duration of his experiments was short enough so that the rates obtained in carbonate mixtures were not influenced by diffusion. The same cannot be said of Arnold's experiments, and Romell's discussion makes it clear that no measurements of rate of assimilation in standing fluid can be depended upon to be uninfluenced by external diffusion of carbon dioxide, unless it is clearly shown, as in the case of James's experiments, that such diffusion is not a factor.

Bukatsch (1935) made experiments on water plants closely similar to those of Harder and Arnold, except that his vessels were attached to a rotating wheel during exposure to light. He records no experiments to show that the mixing achieved in this way was adequate to eliminate diffusion of carbon dioxide, but his curves do not show the decline in rate found by Arnold and Harder, and the initial rise is of relatively short duration. Certainly if diffusion played a part in his experiments it was smaller than in those of Arnold and Harder.

Considering the experiments so far discussed, and other similar ones of which no mention has been made, the writer would state the case as follows: Green plants exposed to strong light may show a loss of chlorophyll and a disappearance of starch. Frequently there is a measurable decrease in rate of carbon dioxide assimilation. This may take place without apparent loss of chlorophyll. There are no recorded cases where depression of assimilation has been quantitatively connected with loss of chlorophyll, but it is conceivable that photo-oxidative destruction of chlorophyll might be responsible for reduced photosynthesis in strong light. It also appears possible that inadequate carbon dioxide provision may sometimes be responsible for the fall in rate.

The experiments with *Chlorella* now to be described will make possible a more coherent statement than the above. The culturing of the cells and the manometric measurements of rate of photosynthesis were carried out as described by Warburg (1928) with minor modifications introduced by the writer (1929). During the rate measurements, the cell suspensions are shaken at a rate which has been found by experiment to provide carbon dioxide and oxygen exchange unlimited by diffusion. Never in the course of hundreds of experiments has the writer found a

decline in rate attributable to high light intensities, though this effect has often been looked for, and light intensities up to about 100,000 meter candles have been used. However, the measurements can be made in 15 minutes. The writer published in 1929 a record of an experiment showing constancy of rate for one hour (p. 615), but as far as he is aware no figures have hitherto been published for longer exposures. In the experiments to be described here, cell suspensions were exposed for 4 to 24 hours, either in closed vessels containing a high initial concentration of carbon dioxide, or in vessels so constructed that a stream of carbon dioxide in air could be passed over the suspension continuously during exposure to light. Illumination was provided by a row of 100-watt internally frosted incandescent lamps spaced 7 cm. apart on centers and located 8 cm. below the bottoms of the vessels containing the cell suspensions. Making the simplifying assumption that each bulb is a point source of light, and taking the manufacturer's figure of 120 candle power per bulb, the intensity at the vessel is estimated at approximately 45,000 meter candles. This corresponds to the intensity of moderate sunlight, and is about 14 times as great as the intensity at which the cells are cultured.

In Table I are collected the results of four experiments in which cell suspensions were exposed to light for about four hours. The rates of assimilation at the beginning and end of exposure are given in the last two columns, in $\text{mm. oxygen per hour per cmm. cells}$ initially filled into the vessels. The cells suspended in culture medium grow during the experiments, and it is not surprising that the rate of assimilation is somewhat higher at the end than at the beginning of exposure. The cells suspended in phosphate do not grow during the experiments, but the rate of assimilation will not stay constant indefinitely in this medium, as shown by the second horizontal row of figures. The 25 percent decline in rate is the result of injury by the phosphate solution, and not by the exposure to light, as is made clear by figures for cells suspended in culture medium. Efforts to find a medium lacking essential elements for growth which would support a constant rate of photosynthesis for many hours have been unsuccessful. For this reason in the remaining experiments phosphate was used as a suspending fluid only when the exposure times were short enough so that there was no appreciable fall in rate, as in the 1st horizontal row of figures in Table I, and for longer exposures, culture medium was used. The extent of increase due to growth is always shown by a control vessel.

To test the effect of standing fluid as a barrier to carbon dioxide provision for assimilating cells, two equal portions of cell suspension were ex-

TABLE I

Suspending fluid	Method of carbon dioxide provision during exposure	Carbon dioxide conc.	Volume of cells used for rate measurement	Rate of Assimilation	
				At beginning of exposure	At end of exposure
		<i>Vol. per cent</i>	<i>mm.</i>	<i>mm. O₂</i>	<i>mm. O₂</i>
M/25 KH ₂ PO ₄	* Vessel closed with high initial concentration	6.7	2.2	18.6	19.4
		4.5	6.5	23.6	17.5
Culture Medium	Gas mixture passed through vessel continuously	6.7	6.5	24.1	25.4
		6.7	10.0	29.7	38.5

Constancy of rate of photosynthesis at beginning and end of four-hour period of exposure to strong light. Rates are given in cmm. oxygen per hour per cmm. cells initially filled into vessels. Temp. 20 degrees C.

* The amount of carbon dioxide used during the exposure was about one half the amount initially present, so that the concentration never fell below the saturating level.

posed to strong light for four hours, in vessels through which a stream of air containing carbon dioxide was passed continuously during the exposure. The gas stream passed over the surface of the cell suspension, and was not bubbled through the fluid. One vessel was shaken in the usual way during the exposure, while the other remained unshaken, clamped in position at the same distance from the light source as the shaken vessel. The rate of photosynthesis was measured before and after exposure, both vessels being shaken in the usual way with a carbon dioxide concentration of 6.7 percent in air. This concentration, which is well above saturation, was also passed through the vessel which was shaken during exposure. The mixture passing through the unshaken vessel during exposure was 0.5 percent carbon dioxide in air. This is also above saturation when adequate shaking is provided.

The results of this experiment are shown in Table II, where the rates of assimilation before and after exposure are given for the shaken and the unshaken suspension.

When proper carbon dioxide provision is restored to cells which have been in stagnant fluid for four hours, the rate of assimilation has fallen to about 65% of the initial rate, and about 50% of the rate of the control after exposure.

It might be objected that the carbon dioxide concentration passed through the unshaken vessel during exposure (0.5 percent) was much lower than in the control (6.7 percent), and that the shaken vessel might also have shown injury, had it been exposed with 0.5 percent carbon dioxide. An experiment showed that this was not the case. For a cell suspension shaken during exposure, the final rate of assimilation is the same whether 0.5 or 6.7 percent carbon dioxide has been used dur-

TABLE II

Treatment of vessel during exposure	Conc. of carbon dioxide in gas stream passing through vessel during exposure	Initial volume of cells	Rate of Assimilation	
			Before exposure	After 4 hr. exposure
	<i>Vol. per cent</i>	<i>mm.</i>	<i>mm. O₂</i>	<i>mm. O₂</i>
Shaken continuously	6.7	10	29.7	38.5
At rest	0.5	10	29.1	20.7

The effect of lack of shaking during exposure to light. Cells suspended in culture medium, at 20 degrees C. Rates of assimilation are in cmm. oxygen per hour per cmm. of cells initially filled into vessels.

ing the exposure. For technical reasons, and because of limited equipment, it was not possible to carry out the entire experiment in 0.5 percent carbon dioxide. This concentration was selected for the unshaken vessel because it was known to give adequate carbon dioxide provision for shaken suspensions, and at the same time approached more nearly the conditions used for example in Harder's experiments, where standing fluid initially in equilibrium with ordinary air was the medium used to provide carbon dioxide for assimilating material. We know from other manometric experiments not reported here that even for shaken cell suspensions, a current of ordinary air does not give adequate carbon dioxide provision. The figures in Table II show that if we use standing fluid, as was done by Harder, and introduce the improvement of a stream of gas containing a much higher carbon dioxide concentration than ordinary air, there is nevertheless a considerable injury to the assimilatory mechanism, revealed when proper shaking is restored, and photosynthesis is compared with cells exposed to light under conditions known to give adequate carbon dioxide provision.

That the injurious effect shown in Table II was due to shortage of carbon dioxide rather than to other effects of stagnation was shown by

shaking samples of cells in the light without providing any carbon dioxide. Table III shows the results of two such experiments, the first with cells suspended in phosphate, the second with cells in culture medium. Alkali was used in the insets of vessels where no carbon dioxide was provided, to absorb the carbon dioxide initially present in the air, as well as any that might be liberated in respiration.

Two controls were used for the experiment in phosphate, one with carbon dioxide and exposed to the light, the other without carbon dioxide, but kept in darkness during the exposure time. The first horizontal row in Table III is for the control with carbon dioxide in light, the second row, marked *Dark* in the column of carbon dioxide concentrations, is for the control without carbon dioxide in the dark. The third row shows the experiment, with cells exposed to light in the absence of carbon dioxide. These cells show a reduction in assimilation of about 50 percent as a result of the exposure. The two controls show conclusively that the injury is due to the combined effect of illumination and carbon dioxide starvation. As explained in connection with Table I, cells suspended in phosphate and exposed to light in the presence of carbon dioxide show a decrease

TABLE III

Expt. No.	Suspending fluid	Initial cell volume	Duration of exposure	Method of carbon dioxide provision during exposure	Carbon dioxide conc. during exposure	Rate of Assimilation	
						At beginning of exposure	At end of exposure
		<i>emm.</i>	<i>hours</i>		<i>Vol. per cent</i>	<i>emm. O₂</i>	<i>emm. O₂</i>
1	M/25 KH ₂ PO ₄	3.5	5	Vessel closed at high initial concentration	6.7	33.8	27.4
					<i>Dark</i>		
					0		35.6
					0		14.7
2	Culture Medium	10	16	Gas stream passed through vessel continuously	6.7	30.9	68.5
					0		9.6

The influence of strong light in the absence of carbon dioxide. Temp. 20 degrees C. Rates of assimilation were measured in 6.7 percent carbon dioxide, and are given in *emm.* oxygen per hour per *emm.* of cells initially filled into vessels.

in rate due to lack of essential nutrient salts. It is interesting to note that the deleterious action of the phosphate medium does not appear in the dark control.

Experiment No. 2 in Table III is a similar one carried out in culture medium instead of phosphate, and exposed for sixteen hours instead of five. No dark control was used here. During the sixteen-hour exposure, the control cells in the light with carbon dioxide more than doubled their rate of photosynthesis, while in the experiment without carbon dioxide, the same exposure to light caused about a 70 percent decrease in assimilation.

Attempts to determine the nature of the injury resulting from exposure to light in the absence of carbon dioxide have given only negative results. Some of these seem of sufficient interest to merit mention here.

The extent of injury by light in the absence of carbon dioxide is the same in air as in commercial nitrogen (oxygen content about 1 percent). Even in oxygen-free nitrogen, the cells would presumably not be entirely deprived of oxygen, since we must suppose that the carbon dioxide set free in respiration is immediately reduced by the photosynthetic mechanism. But under the conditions of these experiments, the respiration of

Chlorella is considerably inhibited by commercial nitrogen. If the injury to the photosynthetic mechanism is oxidative, then it is clear that only very small amounts of oxygen are involved. In the experiments in air without carbon dioxide, no measurable quantities of oxygen were used during the light exposure.

Reduction of the temperature from 20 to 10 degrees C. does not alter the onset of injury in any obvious way.

Comparisons have been made of the extent of the injury in different colors of light, in an effort to show whether chlorophyll acts as a sensitizer for the effect.

Cells deprived of carbon dioxide were exposed to ordinary incandescent light as usual, and to the same light filtered through Corning "Traffic Red" glass HR 245, transmitting up to about 620 m μ , and Corning "Sextant Green" glass No. 401, transmitting from about 580 to 480 m μ . The red glass transmits light strongly absorbed by chlorophyll, and permits maximum photosynthesis when used in conjunction with 100-watt incandescent lamps. The green glass, transmitting light which is hardly absorbed by chlorophyll, gives under the same conditions no measurable photosynthesis above the compensation point. Nevertheless, injury to the assimilatory capacity of the cells is

TABLE IV

Expt. No.	Suspending fluid	Initial cell volume	Duration of exposure	Gas space during exposure	Lighting during exposure	Rate of Assimilation	
						Before exposure	After exposure
		cmm.	hours			cmm. O ₂	cmm. O ₂
				6.7% CO ₂			21.9
1	M/25 KH ₂ PO ₄	1.9	3½	Air without CO ₂	Full incandescent light	No determination made	11.2
				Nitrogen without CO ₂			11.6
2	Culture Medium	60	6	6.7% CO ₂	Full incandescent light	41.3	60.2
				Air without CO ₂			16.7
					Red glass		17.8
					green glass		23.3

Experiments to show the effects of nitrogen and of different colors of light upon the depression in assimilation resulting from exposure to light in the absence of carbon dioxide. Temp. 20 degrees C. Rate measurements made in 6.7 per cent carbon dioxide, and expressed as cmm. oxygen per hour per cmm. of cells initially filled into vessels.

nearly as great in the green as in the red light. This makes it appear unlikely that chlorophyll acts as a photosensitizer in producing the injury. Table IV shows some of the results obtained in different colors of light, and in nitrogen as compared to air.

When the assimilatory rate for normal cells in high and low light intensity was compared with the rate for cells injured by lack of carbon dioxide, it was found that both kinds of cells showed the same relative changes in assimilation with light intensity. Consequently it cannot be said that the injury influences either the Blackman or the photochemical reaction specifically.

Noack (1925) suggested, partly as a result of his studies on the chlorophyll-sensitized photo-oxidation of benzidine, that when green plant cells are deprived of carbon dioxide as an acceptor for the radiant energy absorbed by chlorophyll, then the pigment itself will be photo-oxidized. His efforts to demonstrate this on living material were unsuccessful, and appear to have been without quantitative determinations of either chlorophyll or rate of assimilation, but he nevertheless stated that the decline in assimilation observed in strong light was attributable in part to photo-oxidation of the chlorophyll. This hypothesis was tested for *Chlorella* by making chlorophyll determinations on samples of cells before and after exposures to light in the absence of carbon dioxide, the exposure times chosen being sufficient to reduce the rate of assimilation forty percent or more. A decrease in chlorophyll content equivalent to or even approaching the fall in rate of assimilation was never found. After exposures of less than 6 hours there was no measurable change in chloro-

phyll content whatever. After very long exposures, when the rate of assimilation had fallen to about 30% of the initial rate, the chlorophyll content had only fallen to about 80% of the initial value. Table V shows some figures for chlorophyll content before and after a 16-hour exposure to light with and without carbon dioxide. During exposure, the cells were suspended in culture medium, and it is interesting to note that there was no change in the volume of the cells deprived of carbon dioxide during exposure, while those provided with carbon dioxide nearly trebled in volume. In neither case was there any change in chlorophyll content sufficient to account for the observed changes in rate of assimilation, which are shown in Experiment 2 of Table III. The cells which increased in volume more than doubled their assimilation, in spite of the fact that there was no accompanying change in chlorophyll content. The cells deprived of carbon dioxide, and showing no change in volume, lost only about 20% of their chlorophyll, but 70% of their assimilatory capacity. It may be that distributing a given amount of chlorophyll among a larger volume of cells improves its effectiveness, though it is also possible, and perhaps more likely, that the increased assimilation is due to increased capacity for the Blackman reaction.

It might appear that the fall of 20 percent in the chlorophyll content of the cells exposed to light without carbon dioxide should lend some support to Noack's theory that injury to assimilation under these conditions is due to photo-oxidation of the chlorophyll. However, the writer is not of this opinion because, as mentioned above, experiments with four instead of sixteen hours'

TABLE V

Treatment of sample before extraction	Initial volume of cells in sample	Volume of cells at time of extraction	Chlorophyll in mols x 10 ⁸	
			per cmm. cells	total for sample
	<i>cmm.</i>	<i>cmm.</i>		
Extracted without exposure	10	10	1.44	14.4
Exposed to light with CO ₂	10	28	0.52	14.7
Exposed to light without CO ₂	10	10	1.21	12.1

Changes in cell volume and chlorophyll content of cells exposed to light for 16 hours in culture medium with and without carbon dioxide. Figures for the assimilation rates of these cells are shown in Experiment 2, Table III.

exposure showed a decrease of nearly 50 percent in assimilation, and no measurable change in chlorophyll content whatever. It may be mentioned that Mevius (1935), experimenting with the behavior of the pigment of leaves in the absence of carbon dioxide, also failed to confirm Noack's belief in the photo-oxidation of chlorophyll under these conditions.

Summary and Discussion

Perhaps it should not be said that the rate of photosynthesis in *Chlorella* is ever truly constant, although evidently for periods of one to four hours it shows only minor changes. Over longer periods of time there appears a definitely measurable increase in rate if the cells are suspended in culture medium. The increase in assimilation is accompanied by a roughly corresponding increase in cell volume. If the cells are suspended in phosphate solution, or some other medium lacking elements essential for growth, there is no increase in cell volume, and a decline in rate of assimilation is evident. This decline may be present from the start, but during the first few hours it is usually so small as to escape detection with our methods of measurement. It is certainly not brought on solely by the high light intensity to which the cells are exposed, because no depression of assimilation occurs if cells are exposed to the same conditions in culture medium. Bearing in mind these limitations, then, we may say that the rate of photosynthesis in *Chlorella* is, for all practical purposes, constant under constant external conditions. A light intensity 14 times as great as that to which the cells are accustomed causes no injurious effects provided the carbon dioxide supply remains adequate.

If the supply of carbon dioxide is shut off, either by removing it from the gas space with alkali or by allowing cell suspensions to stand unshaken in the strong light, injury to the assimilatory mechanism results. This injury is not necessarily accompanied by any loss of chlorophyll whatever. It seems to be independent of oxygen partial pressure, at least down to the concentration in commercial nitrogen (about 1 percent). The evidence at hand so far indicates that chlorophyll is not the photosensitizer for the injury.

The writer suggests that it is not improbable that the frequently observed decline in assimilatory capacity during exposure to strong light may be due to inadequate provision of carbon dioxide, rather than to the complexes of internal factors which have been proposed to explain the reported inconsistencies in rate under supposedly constant external conditions. It has already been pointed out that in those cases where a declining rate has been found, the adequacy of the carbon dioxide provision is at least questionable. Where the ex-

perimental technique has been such that good provision of carbon dioxide would be expected, no marked decline in rate of assimilation has been found. The situation seems pretty clear in the case of water plants, where a want of circulation can play a decisive part. For leaves, where the gas exchange is regulated by stomata, it is more difficult to judge whether in the various cases the provision of carbon dioxide has been adequate, but the available evidence suggests that partial closure of the stomata may often have so obstructed the entry of carbon dioxide into the intercellular spaces that the cells or plastids more distant from the source of supply may have suffered the same sort of injury as has been demonstrated for *Chlorella*. Miss Schoder (1932), in discussing the influence of external conditions on the diurnal course of assimilation in leaves, mentions some cases where the rate declined without accompanying related changes in external factors, and notes that when the temperature and light intensity were unusually high, and photosynthesis fell below the compensation point, the stomata were observed to be nearly closed. She interprets the injury as due to heating, as a result of reduced transpiration. But we have Holman's evidence, already described, that solarization may take place independent of any heat effect and also his finding that solarization could be greatly delayed or prevented entirely by raising the carbon dioxide concentration. Admittedly the connection between solarization and depressed assimilation may be only indirect, but Holman's result is just what we should expect if the proposed interpretation is true.

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DISCUSSION

Dr. van Niel: In order to establish the function of chlorophyll in solarization, experiments would be necessary in which such intensities of light of various wavelengths are used that comparable quantities of energy are absorbed. Then the results produced by these on the cells in the absence of CO₂ can be compared directly.

Dr. Emerson: Yes, such experiments are necessary before we can say with certainty whether chlorophyll is the sensitizer involved in the injury. The experiments with red and green light reported here can only be taken as an indication.

Dr. Arnold: With the exception of the method of causing the injury, these effects parallel the results with ultraviolet radiation.

Dr. Emerson: Nevertheless I think the character of the injury may prove to be different in the two cases.

Dr. Harris: What is the basis upon which you make your choice in favor of enzymatic changes rather than changes in the effectiveness of the chlorophyll?

Dr. Emerson: We are dealing here with cells whose assimilatory mechanism is light-saturated. This is interpreted to mean that the rate is limited by the dark chemical processes rather than by the photochemical reaction. If the chlorophyll system, which we suppose determines the capacity for the photochemical reaction, is light-saturated both before and after the growth of the exposed cells, and contains in each case the same total amount of chlorophyll, then we can say with fair certainty that the capacity for absorbing light cannot be limiting in either case, and by inference, the injury must be to other parts of the mechanism than the photochemical reaction. It could be objected that the effectiveness of the chlorophyll system may not depend exclusively on the total chlorophyll content. This may be so, but the capacity for light absorption must be supposed to depend directly on the amount of chlorophyll present.

Dr. Inman: In your definition of solarization injury, does the retardation of photosynthesis take into account the possible injury to respiration?

Dr. Emerson: Injury to respiration was not considered. Respiration corrections, though not used in this work, would not alter the essential character of any of the results. They constitute a negligible fraction of photosynthesis in these experiments.

Dr. Inman: It seems to me that high light intensity that is 20 to 30 times the intensity at which the plant normally grows might injure respiratory processes. Have you measured respiration after exposure to such intense light?

Dr. Emerson: We have many times. High light intensities have never given any indication of large changes in the subsequent dark respiration.

Dr. Inman: *Chlorella* cells do not usually show uniform chlorophyll content, do they?

Dr. Emerson: In thin homogeneous cultures, the cells look pretty uniform under the microscope.

Dr. Inman: How much do they vary in diameter?

Dr. Emerson: Probably from about 3 to 5 μ .

Mr. Lineweaver: The dependence of the injury on absence of CO₂ is, of course, comparable to the instability of certain enzymes (invertase, proteinases, and others) in the absence of their substrates.

An attack on the suggestion that the Blackman factor has been injured would seem possible by the use of flashing light. The Blackman reaction has been interpreted as the enzymic decomposition of the product of the light reaction. If the relative rates in continuous light reflect the relative amounts of Blackman enzyme, after the exposure one should by this interpretation observe about a 10 fold difference in the dark time required to give half maximum yield.

Dr. Emerson: This would be another way of testing for injury to the Blackman reaction.

Dr. Mestre: I think you should get a certain simplification of the experiment if you started with cells already adapted to intense illumination.

Dr. Emerson: One would gain by using cells whose chlorophyll content would not change during the experiment, but one would still have the high rate of growth with which to contend.

Dr. Brackett: We conducted experiments, imperfect in form, in which plants were grown with and without infrared but approximately equal visible intensity. Plants grown with infrared radiation showed marked increase in wet weight and decrease in dry weight. I am wondering to what extent part of the growth observed in your experiments is merely increase in water content.

Dr. Emerson: Dry weight determinations have not yet been made with these cells, but it is likely that such rapid growth is due largely to water intake.

Dr. Rothmund: I would like to know the temperature at which the chlorophyll in the living plant is damaged.

Dr. Mestre: The temperature at which destruction takes place will of course vary in different plants. Typically, if the internal temperature of the chromatophore remains at 55° C. for 5 minutes, there would be irreversible changes in the chlorophyll system demonstrable spectrographically. The same spectral changes can be demonstrated at 50°, but the reaction is slower, as the process follows the Arrhenius equation, with a temperature characteristic of 80 to 100 thousand.

Mr. Lineweaver: Would that damage depend on the carbon dioxide concentration?

Dr. Mestre: That I cannot answer. In these experiments the leaves or pieces of algae were dipped into a thermostated water bath for definite periods of time, immediately cooled, and the absorption spectrum determined.

Mr. Lineweaver: If *Chlorella* cells, in the presence of optimum light intensity, were exposed to some other injurious factor that depends on time (e.g., temperature, pH, etc.) would the injury to the photosynthetic activity depend on the presence or absence of CO₂?

Dr. Emerson: Experiments of this kind with other methods of injury have not been made.

Dr. Mestre: Were any determinations made of the absorption spectrum of the cells after exposure to light?

Dr. Emerson: No, no such determinations have been made.

Dr. Mestre: The reason I ask is because there might be changes in the condition of the pigments which would render them nonfunctional for the assimilation process, without producing any de-

tectable change in the extracted pigments. This will be further discussed in my paper on the photosynthetic system of the chromatophore.

Dr. Zscheile: Is it possible that, during injury in solarization, inactivation of an enzyme of the photosynthetic system might occur as a reaction photosensitized by chlorophyll? In the presence of CO₂, the energy absorbed by chlorophyll would be partially utilized in photosynthesis. In the absence of CO₂, perhaps some of this energy would be used in promoting inactivation of an enzyme factor. At higher light intensities, this inactivation would be more rapid. It would necessarily be a very inefficient photosensitized reaction, on the basis of the entire visible radiation absorbed.

Dr. Emerson: If you assume that the green light absorbed by chlorophyll, though of small energy content compared to the red which is absorbed, is much more effective than the red in promoting inactivation, then chlorophyll may still be the photosensitizer for the inactivation.

Dr. Giese: Early in the paper you remarked that the bleaching of leaves by light was due to the heating effects of absorbed light. Yet a boiled leaf remains green. How do you explain the apparent discrepancy?

Dr. Emerson: I should conclude from this that light as well as heat plays a part in the injury.

Dr. Kohn: Where within the photosynthetic mechanism would you place the sensitive locus defined by your experiments?

Dr. Emerson: The results obtained so far do not seem to me to warrant any positive statement concerning the part of the assimilatory mechanism which is injured.

PHOTOSYNTHESIS OF BACTERIA

C. B. VAN NIEL

PART I. PHOTOSYNTHESIS AS AN OXIDATION-REDUCTION PROCESS

1. Introduction.

The first evidence for the existence of photosynthetically active bacteria was published in 1883 when Engelmann⁽¹⁾, as a result of physiological studies with certain red-colored bacteria, expressed the view that these organisms were capable of carrying out photosynthesis. Engelmann was struck by the fact that these bacteria accumulated in the spectrum in such a manner as to coincide exactly with their absorption spectrum.

Since that time three widely different views concerning the metabolism of these "purple bacteria" have been postulated, and for each one experimental evidence was adduced.

A. Engelmann's idea of photosynthetic activity was supported by the phototactic behavior of the organisms, and also by later experiments which showed that no development occurs except in the presence of light.

Against this hypothesis was the fact that no one has ever succeeded in demonstrating beyond a doubt the production of oxygen during illumination. Engelmann's seemingly positive results of 1888⁽²⁾ have been refuted by later work in which a more sensitive, as well as more conclusive, method gave absolutely negative results. (Molisch⁽³⁾, Muller⁽⁴⁾, Inman⁽⁵⁾). Another strong argument against photosynthetic activity was the necessity of hydrogen sulphide or organic matter.

B. The necessity of hydrogen sulphide was used as an argument for the second hypothesis, which has maintained itself by the side of the previous one. This was advanced in 1887 by Winogradsky⁽⁶⁾, who had discovered the existence of bacteria capable of development in the dark with carbon dioxide as the only carbon source. The first example of such organisms were the (colorless) sulphur bacteria which ox-

dize hydrogen sulphide and sulphur in the dark to sulphuric acid. They not only maintain themselves on the energy derived from this oxidation, but are also capable of reducing carbon dioxide in the dark at the expense of this energy (chemosynthesis). This same conversion of hydrogen sulphide and sulphur to sulphuric acid was also observed with the purple bacteria, and thus the idea that these organisms would be chemosynthetic was advanced.

Undoubtedly the fact that H₂S seemed an essential factor for development was a strong argument in favor of this theory. Yet, it did not explain why light was also necessary. Another weak point was the decidedly anaerobic character of the organisms, because oxygen would be necessary for the oxidation of H₂S to H₂SO₄.

C. In 1907 Molisch⁽³⁾ brought forward evidence to show that H₂S is not necessary, but that growth takes place only in the presence of organic matter. Actually pure cultures of purple bacteria were obtained which could be grown as ordinary saprophytes in the dark with organic substances.

Molisch's work has later led to a subdivision of the purple bacteria into two groups, the Thiorhodaceae, comprising those organisms which develop in a mineral medium in the presence of H₂S, and the Athiorhodaceae, which require organic substances. Although the occurrence of a pigment system containing red and green pigments is characteristic of both groups, a close physiological relationship was deemed improbable by later workers.

2. Photosynthesis of green and purple sulphur bacteria.

These three hypotheses have existed side by side until 1929. Quantitative studies, carried out with a number of pure cultures, then made possible a synthesis of these divergent viewpoints⁽⁷⁾.

The experimental results of these studies can be summarized as follows:

1. There exist bacteria which can develop in entirely inorganic media containing H₂S, in the complete absence of oxygen, but only in the light.
2. No development of these organisms takes place if H₂S is omitted.
3. In media containing a sufficient quantity of NaHCO₃, ammonia-N, K, P, and Mg the amount of development is strictly proportional to the quantity of H₂S present.

⁷ van Niel, C. B., in *Contrib. to Marine Biology*, Stanford Univ. Press, 161, 1930.

¹ Engelmann, Th. W., *Arch. ges. Physiol.*, **30**, 90, 1883.

² Engelmann, Th. W., *Arch. ges. Physiol.*, **42**, 183, 1888; *Bot. Ztg.* **46**, 661, 1888; *Arch. Neerland.*, **23**, 151, 1888.

³ Molisch, H., *Die Purpurbakterien nach neuen Untersuchungen*, Jena, 1907.

⁴ van Niel, C. B., and Muller, F. M., *Rec. trav. bot. néerl.*, **28**, 245, 1931; Muller, F. M., *Arch. Mikrobiol.*, **4**, 131, 1933.

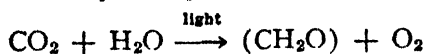
⁵ Inman, O. L., Personal communication, 1933.

⁶ Winogradsky, S., *Bot. Ztg.*, **45**, 489, 1887; *Beiträge zur Morphologie und Physiologie der Bakterien. Heft I. Zur Morphologie und Physiologie der Schwefelbakterien*, Leipzig, 1888.

4. No development takes place in the absence of CO₂ (carbonate, bicarbonate).
5. Oxygen is not produced.
6. During the development of these organisms H₂S becomes converted into S (green bacteria) or into H₂SO₄ (Thiorhodaceae).
7. The reaction of the medium becomes more and more alkaline due to disappearance of CO₂.
8. Chemical analyses show that there exists a stoichiometrical relationship between the quantity of H₂S oxidized and the amount of CO₂ which has disappeared, to wit: for one molecule of H₂S oxidized to S, 0.5 molecule of CO₂ disappears (green bacteria); for 1 mol. of H₂S oxidized to H₂SO₄ almost 2 mol. of CO₂ (1.8) disappear.
9. The carbon of the CO₂ which has disappeared can be recovered as organic carbon in the form of bacterial substance.
10. In the dark, in the absence of oxygen, no development takes place; H₂S is not converted into S or H₂SO₄, and there is no disappearance of CO₂.

The biological conversion of CO₂ into organic matter dependent upon illumination is photosynthesis. Inasmuch as this conversion is carried out by the green and purple sulphur bacteria we must, therefore, consider these organisms photosynthetic.

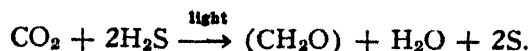
On the other hand, a study of the photosynthetic activity as displayed by the green plants has revealed that fundamentally this process can be expressed by the equation



The absence of any evidence of O₂-production by the bacteria thus seems a considerable obstacle to the acceptance of such a process in these organisms. Yet, if one compares the metabolism of the green bacteria—and also the early stages of the metabolism of the Thiorhodaceae in H₂S containing media, during which there is a rapid disappearance of H₂S while the cells store up elementary sulphur—with photosynthesis of the green plants, the relationship at once becomes more clear. For the quantitative connection between the participants in the metabolism of the bacteria can be expressed by the equation:



This equation is a first approximation and implies a composition of the bacteria corresponding to that of a carbohydrate. But then one might also write this equation as follows:

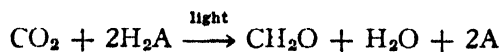


In doing so the above-mentioned relation can be paraphrased in the following terms: whereas in the green-plant-photosynthesis H₂O, interacting with CO₂, gives rise to O₂-production, the bacterial photosynthesis, starting with H₂S instead of H₂O, leads to S.

It is well known that in 1913 H. Wieland began to bring forth evidence in favor of the viewpoint that the mechanism of respiration processes can ultimately be reduced to a dehydrogenation of the substrate⁽⁸⁾. In 1925 this idea was extended by Kluyver and Donker to cover all metabolic processes⁽⁹⁾. The most general form in which a metabolic process might then be expressed is



On the basis of this hypothesis the two types of photosynthetic reactions can then be regarded as special representatives of a generalized photosynthetic process:



The experimental evidence so far presented in connection with the metabolism of the green and purple sulphur bacteria fits in with this formulation which implies that for different photosynthetically active organisms different hydrogen donors for the final reduction of CO₂ may be required.

This formulation also shows at least the possibility of the occurrence of photosynthetic processes in which the hydrogen donor H₂A is neither H₂O nor H₂S.

A number of such possibilities have been verified. I mention here e.g. photosynthesis of the Thiorhodaceae in the presence of Na₂SO₃, Na₂S₂O₃, elementary sulphur⁽¹⁰⁾, and with molecular hydrogen⁽¹¹⁾. Furthermore, it has been possible to make these bacteria develop in media containing simple organic substances instead of sulphur compounds. Also in this case it seemed possible to explain their metabolism as some sort of photosynthesis (or photochemical CO₂-reduction) in which the organic substance serves as H-donor. This, I am aware, seems far-fetched;

⁸ Cf., e.g., Wieland, H., On the mechanism of oxidation, Yale Univ. Press, 1932.

⁹ Kluyver, A. J., und Donker, H. J. L., Chem. Zelle Gewebe, 13, 134, 1926; Kluyver, A. J., Chemical Activities of Microorganisms, London Univ. Press, 1931.

¹⁰ van Niel, C. B., Arch. Mikrobiol., 3, 1, 1931.

¹¹ Roelofs, P. A., Proc. Kon. Akad. v. Wetensch. Amsterdam, 37, 660, 1934. Also: On photosynthesis of the Thiorhodaceae, Thesis, Utrecht, 1935.

a priori it appears rather improbable that an organism which has organic compounds at its disposal would build up its cell-constituents from CO_2 !

Yet, if we scrutinize the available evidence there is much which speaks in favor of this concept. Muller⁽¹²⁾ was the first to bring together quantitative data relating to this problem. Working with a standard mineral medium to which one single organic substance had been added, and analyzing media prepared in this way, after development of the cultures, he could demonstrate:

1. that a variety of simple organic substances, such as fatty, hydroxy-, keto-, and dibasic, acids will allow of a development of the bacteria in the complete absence of O_2 , but in the light;
2. that at the conclusion of the experiment no organic matter was left in solution in the medium, while the carbon originally present in the added substrate could be recovered as cell-substances (and CO_2);
3. that in some cases, and notably with the higher fatty acids, development was dependent upon the presence of CO_2 in the medium. In these cases the CO_2 -content of the medium decreased, and the total organic carbon in the bacteria exceeded the total carbon in the added organic substance by a quantity practically equal to the carbon of the disappeared CO_2 .

First of all, the last-mentioned fact—the formation of cell-substance from CO_2 —shows conclusively that photosynthesis had taken place. This same phenomenon has later been studied by Gaffron⁽¹³⁾ who, on the basis of measurements with the Barcroft-Warburg technique, reached the conclusion that the Thiorhodaceae are unable to carry out photosynthesis with organic substrates. According to Gaffron the observed facts must be considered as the resultant of two processes. In the first the organic substance is converted into CO_2 with a concomitant reduction of sulphate to sulphide. This process is independent of radiant energy, and therefore takes place in the dark as well as in the light. In the second process the sulphide and CO_2 are then photosynthetically converted into bacterial substance and sulphate.

Although attractive in attributing the ultimate result to the same fundamental process which the Thiorhodaceae exhibit in H_2S -containing media, this explanation has been shown to be erroneous (Roelofsen, (11)). Gaffron did not use pure cultures; his cultures contained sulphate reducing bacteria beside the Thiorhodaceae. After isolating the purple bacterium from Gaffron's cultures

in a pure state, Roelofsen could prove that:

1. neither in the dark nor in the light does this organism reduce sulphates, and that:
2. in a medium in which sulphate is entirely absent organic substances may still be utilized. Hence we must still face the situation as a complete conversion of organic substances of various sorts into cell substance and CO_2 , or of organic substances and CO_2 into cell substance, without the intervention of H_2S .

Since the monumental work of Harden, of Neuberg, and of Kluyver and their collaborators it has become obvious that the most divergent metabolic processes can be resolved into a more or less long series of simple and chemically intelligible step reactions. Kluyver⁽¹⁴⁾ has pointed out on the basis of much experimental evidence that this concept may be applied not only to the so-called katabolic, or breakdown, processes, but also to the anabolic, synthetic reactions.

Obviously, this would mean that also the cells of the purple bacteria are built up gradually, as a result of simple step reactions, and that the reactions leading to this final result are fundamentally the same in various media, no matter what the initial raw material may be. In that case one must needs be able to find a common denominator in all those systems from which the purple bacteria are able to build up their cell constituents.

Now, considering e. g. the series of fatty acids with straight or branched chains, we find that HCOOH , CH_3COOH , $\text{CH}_3\text{CH}_2\text{COOH}$, $\text{CH}_3(\text{CH}_2)_2\text{COOH}$, $(\text{CH}_3)_2\text{CHCOOH}$, $\text{CH}_3(\text{CH}_2)_3\text{COOH}$ etc., are all converted into bacterial cells, and for the members from propionic acid on with the uptake of CO_2 . If it is further borne in mind that the same conversions unquestionably also take place in the systems $\text{CO}_2 + \text{H}_2\text{S}$, and $\text{CO}_2 + \text{H}_2$, then it becomes increasingly evident that the common fundamental reaction for the inorganic systems, i. e. the photochemical reduction of CO_2 takes place as well in those with the organic compounds. And, inasmuch as the only end-product of any consequence in the conversions of simple organic substances—besides bacterial cells—is CO_2 , one is led to the conclusion that just as H_2S or H_2 are dehydrogenated (oxidized) completely, the hydrogen being transferred to CO_2 , so are the organic substances completely dehydrogenated (to CO_2 and H_2O) with CO_2 as the only acceptor.

This formulation does not, of course, imply that during the breakdown of these organic compounds there may not be formed intermediate products which can serve immediately as raw

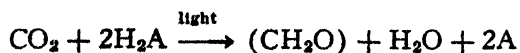
¹² Muller, F. M., Arch. Mikrobiol., 4, 131, 1933.

¹³ Gaffron, H., Biochem. Z. 269, 447, 1934.

¹⁴ Kluyver, A. J., Arch. Mikrobiol., 1, 181, 1930.

material for some of the anabolic reactions. It also does not exclude the possibility that CO_2 is not the only H-acceptor. After all, the organisms contain substances of a more or less highly reduced nature (such as the pigments). Although the mode of formation of such substances is still completely ununderstood, it is quite obvious that the hydrogen necessary for these reductions must come from somewhere. Thus it is entirely reasonable to suppose that some of the dehydrogenations during the oxidation of inorganic and organic substances may take place with some acceptor other than CO_2 .

But the result of these studies has shown that the fundamental photosynthetic process occurring in cultures of Thiorhodaceae in organic media is again one of the general type



in which H_2A , the H-donor, is now represented by simple organic substances.

3. Photosynthesis of the Athiorhodaceae.

The members of this group share with those of the Thiorhodaceae the occurrence in their cells of a pigment complex consisting of green and red pigments which are at least quite similar for both groups. Moreover, the Athiorhodaceae respond to light in the same way as do the sulphur purple bacteria. This holds good not only with respect to their phototactic behavior, but also to their ability to develop in the complete absence of oxygen only when illuminated.

This leads to the question whether one may consider the metabolism of these organisms as essentially similar to that of the Thiorhodaceae. It is true that investigators after Molisch have drawn a sharp line of demarcation between the two groups on account of the fact that the Athiorhodaceae develop only in the presence of organic matter. But may this not mean that these bacteria can use only organic H-donors for the photosynthetic reaction?

It is especially to Gaffron⁽¹⁵⁾ that we owe a considerable amount of information concerning the metabolism of the Athiorhodaceae. This investigator has shown that, in the absence of oxygen, they assimilate carbon dioxide during illumination in the presence of fatty acids. Yet oxygen is not produced, and the amount of CO_2 assimilated is strictly proportional to the quantity of fatty acid present.

This follows also from growth experiments with pure cultures. Such experiments⁽¹⁶⁾ have

established that a variety of simple organic substances are completely converted into cell material and CO_2 , whereas other organic substances can only be so converted with the simultaneous uptake of CO_2 . With compounds of the latter group one can demonstrate that the quantity of organic substrate utilized, and also the cell-yield, is directly proportional to the amount of CO_2 present in the culture medium as long as there is no excess of CO_2 .

In Gaffron's publications one finds conclusive evidence for the statement that the amount of CO_2 assimilated together with one molecule of the fatty acids increases regularly with the length of the carbon chain. Although there exist some discrepancies between the values obtained in different experiments with one and the same substance, and also some minor irregularities, the main trend of these figures is clear, as shown in the following table 1 and in figure 1.

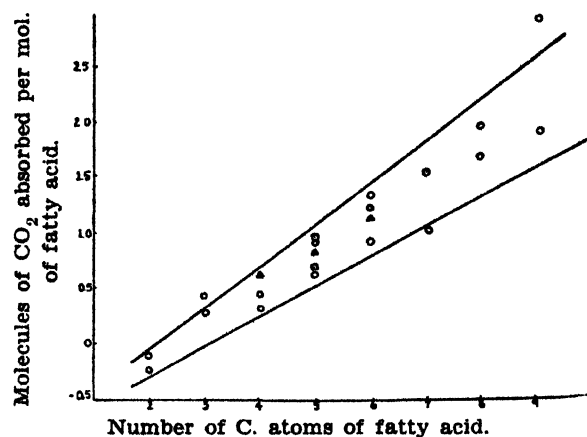


FIGURE 1.

Carbon dioxide assimilation by Athiorhodaceae with fatty acids. Plotted after data by Gaffron.

- Normal acids.
- △ Iso-acids.
- [] Other isomeres.

Ordinates: Molecules of CO_2 absorbed per molecule of fatty acid.

Abscissae: Number of carbon atoms per molecule of fatty acid.

Gaffron's data (3rd column, table 1) were obtained with the Warburg technique.

The figures in the last column are obtained directly from Gaffron's figures by subtracting the quantity of CO_2 absorbed from the gas-phase but remaining in solution as NaHCO_3 .

These data allow one to calculate the quantity of CO_2 assimilated per CH_2 group, and it is clear that this value is approximately 0.4 molecules.

In addition, Gaffron has shown that representa-

¹⁵ Gaffron, H., *Biochem. Z.* **260**, 1, 1933; 275, 301, 1935.

¹⁶ Unpublished results.

TABLE I.

Carbon dioxide assimilation with fatty acids by Athiorhodaceae.
(After Gaffron, 1933-1935)

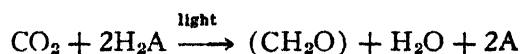
Substance	No. of C. atoms	CO ₂ uptake in moles	
		Total per mol.	Assimilated per mol.
Acetic acid	2	(0.75)-0.89	0.25 - 0.11
Propionic acid	3	1.29 - 1.42	0.29 - 0.42
n.-Butyric acid	4	1.30 - 1.43	0.30 - 0.43
i.- Butyric acid	4	1.61	0.61
n.-Valeric acid	5	1.62 - 1.90	0.62 - 0.90
i.-Butyric acid	5	1.83	0.83
Methyl-ethyl-acetic acid	5	1.68 - 1.91	0.68 - 0.91
n.-Caproic acid	6	1.90 - 2.34	0.90 - 1.34
i.-Caproic acid	6	2.10 - 2.30	1.10 - 1.20
Heptylic acid	7	2.03 - 2.54	1.03 - 1.54
Caprylic acid	8	2.69 - 2.96	1.69 - 1.96
Nonylic acid	9	2.90 - 3.90	1.90 - 2.90

tives of the Athiorhodaceae are also capable of assimilating CO₂ in the presence of molecular hydrogen. In this reaction approximately 2 molecules of H₂ are absorbed together with 1 molecule of CO₂. And, finally, it appears that, in the presence of organic substances and CO₂, H₂S can be used by these organisms in a photosynthetic process in which CO₂ is utilized. With pure cultures of Athiorhodaceae which had been "adapted" to grow in organic media containing H₂S, I have observed an oxidation of H₂S to elementary sulphur, in which per 2 molecules of H₂S oxidized approximately 0.5 mol. of CO₂ was converted into bacterial cells (16). Gaffron's figures for CO₂ uptake make it seem probable that in his experiment the H₂S was completely oxidized to H₂SO₄ with a simultaneous uptake of 2 molecules of CO₂ per molecule of H₂S. However, since the results were obtained with different organisms and under different conditions, and especially since Gaffron did not use pure cultures it is as yet impossible to explain these apparent discrepancies.

This much is certain, that physiologically there exists so great a similarity between the behavior of the Athio- and the Thiorhodaceae in the light, but in the absence of oxygen, that a more detailed discussion of the results obtained with the former can be omitted. The considerations which led to the conclusion that even with organic substrates the photosynthetic process of the Thio-

rhodaceae consists of a reduction of CO₂ with hydrogen, derived from the organic molecule which acts as H-donor, apply also here.

The preceding discussion thus has shown that the concept of photosynthesis as a typical oxidation-reduction reaction of the general type:



furnishes a satisfactory explanation for the metabolism of the green and purple sulphur bacteria and of the Athiorhodaceae. These organisms must be considered as photosynthetic, requiring, however, unusual H-donors for the photochemical reduction of carbon dioxide.

PART II. COMPARATIVE BIOCHEMISTRY OF PHOTOSYNTHESIS

1. The mechanism of photochemical carbon dioxide reduction

If one tries to understand the meaning of the generalized equation for photosynthesis it becomes clear that all those mechanisms proposed for the photosynthetic reaction which imply the formation of a carbonic acid-chlorophyll complex which is subsequently transformed into a form-aldehyde peroxide are not quite in accordance with the formulation of photosynthesis as an oxidation-reduction process. Such schemes fail to give a satisfactory explanation for the photo-

synthetic process carried out by the green and purple bacteria (See also (4)).

From a unified point of view, as laid down in the generalized equation, green plant photosynthesis should be considered as a reduction of CO_2 with hydrogen obtained from H_2O , and the oxygen produced during illumination as dehydrogenated H_2O .

In December, 1932, a paper appeared by Arthur Stoll⁽¹⁷⁾ in which the same idea was used in an attempt to elucidate the mechanism of green plant photosynthesis. In Stoll's words: "The activated carbonic acid would be acceptor for the active hydrogen of the more highly hydrogenated forms of the chlorophyll molecules".

The dehydrogenated chlorophyll becomes reduced again as the result of a photodecomposition of H_2O , which is present in the form of a chlorophyll hydrate. "Water thus bound up with chlorophyll can be decomposed, with the utilization of transformed radiant energy, into H and OH (H_2O_2)."

The so-called Blackman reaction then is responsible for the decomposition of H_2O_2 , and Stoll concludes: "The oxygen evolved during photosynthesis would, therefore, not originate from carbonic acid, but from decomposed water which, via the hydrated form of chlorophyll, furnishes the hydrogen for the carbon dioxide reduction with the formation of H_2O_2 ."

It is easy to see that these ideas—which are fundamentally similar to those expressed by e. g. Shibata and Yakushiji⁽¹⁸⁾, Willstätter⁽¹⁹⁾, and Franck⁽²⁰⁾—are in essence in agreement with the general formulation.

¹⁷ Stoll, A., *Naturwissensch.*, **20**, 955, 1932.

¹⁸ Shibata, K., and Yakushiji, E., *Naturwissensch.*, **21**, 267, 1933; Yakushiji, E., *Acta Phytochim.*, **7**, 93, 1933.

¹⁹ Willstätter, R., *Naturwissensch.*, **21**, 252, 1933.

²⁰ Franck, J., *Naturwissensch.*, **23**, 226, 1935.

On the basis of the concept of photosynthesis as an oxidation-reduction process, it is obvious that the reduction of the carbon dioxide proceeds stepwise. All oxidation-reduction reactions so far known involve the transference of one, or at most two, H atoms at a time. Such a stepwise reduction implies the formation of intermediate products; what these are, remains an open question. They may be radicals (Willstätter-Haber) or more nearly actual chemical compounds. Stoll postulates formic acid as an intermediate product, and since its formation by direct hydrogenation of CO_2 is so easily conceivable, the suggestion is a tempting one. However, conclusive evidence is completely lacking.

Now the purple bacteria seem to offer a much more favorable object for an experimental attack on this question than do the green plants. It is clear that, if formic acid were an intermediate product in the gradual reduction of CO_2 , it must itself be able to act as an acceptor for hydrogen. However, the addition of formic acid to photosynthetically active plants may easily result in an oxidation of this substance and thus it might serve as a secondary source of CO_2 . Since the purple bacteria do not produce any oxygen it follows that the fate of formic acid added to such cultures can be followed up much more conclusively.

The results of such experiments in which purple sulphur bacteria were grown in media containing H_2S , NaHCO_3 and HCOONa are presented in table II.

The conversion of H_2S to H_2SO_4 involves the transference of 8 H atoms per molecule, requiring the reduction of 2 molecules of CO_2 or 4 molecules of HCOOH to the carbohydrate stage. In the table the quantities of H_2S oxidized to H_2SO_4 , and of CO_2 and HCOOH disappeared have been expressed as milli-equivalents hydrogen.

TABLE II.
Results of analyses of cultures of Thiorhodaceae in media containing H_2S , NaHCO_3 and HCOONa .

Expt. No.	H_2S oxidized to H_2SO_4 in m. equiv. H	CO_2 disappeared in m. equiv. H A.	HCOOH disappeared in m. equiv. H B.	A + B
1.	4.08	2.12	1.76	3.88
2.	3.90	1.92	2.00	3.92
3.	6.55	4.33	1.83	6.26
4.	6.30	2.80	3.15	5.95
5.	5.36	-1.47	7.10	5.63

It is clear that the quantity of CO_2 disappeared in the first 4 experiments is much too small to account for the observed H_2SO_4 -formation. Supplemented by the quantity of HCOOH disappeared there is, however, a close agreement between the amount of H donated by the H_2S and the amount taken up by the two acceptors together. Hence these experiments seem to prove that HCOOH can function, like CO_2 , as a true H-acceptor in purple bacteria photosynthesis.

This would be a strong point in favor of the assumption that formic acid is an intermediate product in photosynthesis, were it not for the result of experiment no. 5. For in that case the quantity of HCOOH disappeared is greatly in excess of that which might be expected even if the formic acid had been the only acceptor. Moreover, this experiment shows an increase in CO_2 . Obviously the organic acid has here acted in accordance with the behavior of other organic substances in cultures of purple bacteria, namely as H-donor. Thus it follows that in none of these cultures may the " HCOOH disappeared" have been due to its action as H-acceptor, but as a secondary source of CO_2 , and even the interpretation of the results of the first 4 experiments in favor of the idea that the formic acid would have acted as an additional, competing H-acceptor is open to doubt.

Also in connection with another important problem concerning intermediate products in photosynthesis the purple bacteria have so far not contributed any experimental evidence. I refer to the so-called "first product of photosynthesis" which by a majority of workers is supposed to be formaldehyde.

One of the main arguments for the formation of this substance as an intermediate product is the fact that from a theoretical point of view it is the most easily conceivable reduction product of CO_2 from which complex carbohydrate—the first *detectable* product in green plant photosynthesis—can be formed. In addition to this, one might mention the remarkable constancy of the photosynthetic quotient, duly stressed by Willstätter and Stoll, although this observation by itself signifies only that non-carbohydrate intermediate products never accumulate.

Gaffron, in 1935, has emphasized the fact that in photosynthesis of the purple bacteria one almost never meets with simple stoichiometrical relationships and concludes:

"It is, therefore, probable that photosynthesis of the purple bacteria involves the cooperation of a larger number of molecules, and that several intermediate reactions occur before the first stable reaction products appear. If one assumes that two "Grundkörper" can be formed in variable

ratio then this suffices to explain the variations in the assimilation-value."

This statement seems to contain an argument against a unified concept of photosynthesis in green plants and in purple bacteria. However, this is not necessarily so, because there are sufficient reasons why one cannot yet compare the results of such studies on the green plants with those on the purple bacteria. The chief cause for this is the enormous difference in the rate of growth. This implies that, while in studies of relatively short duration on the green plants one actually determines the formation of carbohydrate from CO_2 , in such studies with the purple bacteria the determined result is the conversion of constituents of the medium (CO_2 and H_2A) into full grown bacteria. As long as the exact chemical constitution of this "product of CO_2 -assimilation" remains undetermined the measurements cannot be subjected to a detailed interpretation. This situation explains readily

1. that the relationship $2\text{A}/\text{CO}_2$ is not unity, because the final composition of the bacteria does not exactly correspond to carbohydrate;
2. that this relationship is not constant, because it depends upon the difference in composition of the cell material at the end and at the beginning of the experiment.

Although a number of kinetic studies on photosynthesis by purple bacteria have been made, these considerations show the futility of attempts to conclude from them which path photosynthesis takes. But even simple studies on the rate of photosynthesis with various H-donors have not always been interpreted correctly.

Gaffron, for instance, presents data which lead him to the conclusion that photosynthesis with different substrates takes place by different mechanisms. Fig. 2 and 3, taken from his paper, show the changes in pressure due to photosynthesis of purple bacteria with pyruvate and lactate, in an atmosphere of nitrogen with 5% CO_2 (Fig. 2), and in an atmosphere of hydrogen (Fig. 3) respectively.

The conclusion that in N_2 lactate is attacked more rapidly than pyruvate is not justified. For what is measured is the rate of CO_2 uptake by medium and bacteria, and this is not a direct measure of the rate of photosynthesis. The experiment in an atmosphere of N_2 does show conclusively that a given number of molecules of pyruvic acid are photosynthesized in exactly $\frac{1}{2}$ the time necessary for photosynthesis of the same number of lactic acid molecules. This becomes readily understandable if we remember that, on the basis of the dehydrogenation theory of photosynthesis, pyruvic and lactic acid are both dehydrogenated with CO_2 as the final acceptor.

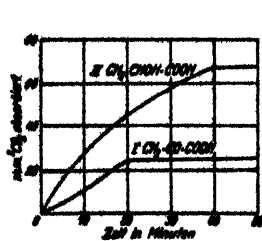


Abb. 2
Absorption von Kohlendioxid durch eine Suspension von belichteten Paprubakterien in Gegenwart von 0,1 cem m/20 Milchsäure und 0,100 cem m/20 Brennstoffsäure

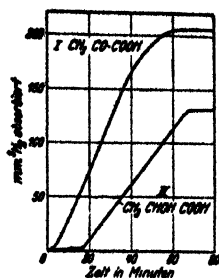


Abb. 3
Reduktion von 0,1 cem m/20 Brennstoffsäure und 0,1 cem m/20 Milchsäure durch belichtete Paprubakterien in Wasserstoff

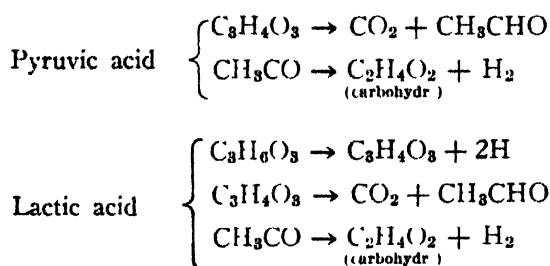
FIGURE II.

Carbon dioxide absorption by Athiorhodaceae. Atmosphere of N_2 with 5% CO_2 . From Gaffron.

FIGURE III

Absorption of hydrogen by Athiorhodaceae in the presence of Na-lactate and Na-pyruvate. From Gaffron.

Because the total quantity of H to be transferred from pyruvic acid in order to reach a certain intermediate stage is also $\frac{1}{2}$ that to be transferred from lactic acid in order to reach the same stage, it appears that the rate of CO_2 reduction is equal in both cases. This may be expressed in the following equations:



These equations are hypothetical, but they serve to demonstrate the principle. They also show that in the case of lactic acid more CO_2 will be taken up by organisms + medium, and calculations show that the quantities found by Gaffron agree very closely with the calculated amount.

In H_2 the situation seems reversed. But also this finds a ready explanation on the basis of the theory developed here. It is very probable that the first stage in the decomposition of pyruvate is a decarboxylation, leading to CO_2 and CH_3CHO . Thus, for the subsequent dehydrogenation of CH_3CHO , CO_2 is available as acceptor. Since for this dehydrogenation only $\frac{1}{2}$ molecule of CO_2 is needed, the remaining $\frac{1}{2}$ molecule can now be used as acceptor for H derived from the molecular H_2 in the gas phase.

On the other hand, the lactic acid will most likely first be dehydrogenated to pyruvic acid. For this dehydrogenation CO_2 (or some other

acceptor) is needed. But the experiment in this case is carried out in the absence of CO_2 . This explains the lag; for hydrogen uptake cannot proceed but in the presence of excess CO_2 . It is only after some time that sufficient extra CO_2 is obtained from the dehydrogenation of the lactic acid—due to the fact that the final composition of the bacteria corresponds to a higher reduction stage than carbohydrate—and only from that time on does H_2 -uptake start at a reasonable rate.

Also Roelofsens (11²) has drawn erroneous conclusions concerning the rate of photosynthesis with different donors. Fig. 4 shows his results expressed a CO_2 -uptake in media containing Na_2SO_3 and H_2S . He states:

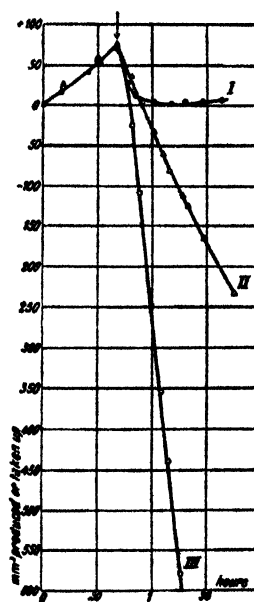


fig. 18.

Carbon dioxide assimilation Na_2SO_3 as a hydrogen donor, in comparison with the auto-assimilation and with the carbon dioxide assimilation with H_2S as a hydrogen donor

Curve I. CO_2 production and CO_2 uptake of ≈ 200 mm³ bacteria of strain a, cultivated in the inorganic medium, exposed to a period of darkness of 20 hours at 38°C. and suspended in tap-water with 3% NaCl and 0.5% $NaHCO_3$ in equilibrium with Na5% CO_2 at 30°C. Velocity of CO_2 assimilation 65 mm³/hour Differential manometer No. 1

Curve II. Same arrangement as in I, except the addition of 0.3% Na_2SO_3 7 NaO Assimilation velocity 370 mm³/hour Differential manometer No. 1

Curve III. Same arrangement as in I, except the addition of an aqueous solution of 3000 mm³ H_2S . Assimilation velocity 1200 mm³/hour Differential manometer No. 1.

FIGURE IV.

Carbon dioxide absorption by Thiorhodaceae in the presence of Na_2SO_3 and of $NaHS$. From Roelofsens.

"The assimilation of CO_2 with an excess Na_2SO_3 as an H-donor proceeded at a well measurable rate, though much slower than with H_2S under the same conditions." (p. 81).

"The CO_2 assimilation with $Na_2S_2O_3$ proceeds nearly as quick as that with Na_2SO_3 under similar conditions." (p. 81).

"The assimilation with S as a donor was too slow to permit its study by the manometric method. . . . Up to the present the remarkable difference in oxidation velocity of H_2S and S had escaped notice." (p. 79-80).

As in the case of Gaffron, Roelofsens mistakes the rate of CO_2 uptake by bacteria plus medium for the rate of photosynthesis. A recalculation

of his data shows, however, that the rate of CO_2 reduction is exactly the same, no matter whether H_2S or H_2SO_3 is the H-donor. The "assimilation velocity" with Na_2SO_3 is 370 cmm. per hour. In this case the reaction product is Na_2SO_4 : the medium does not become alkaline, hence the entire quantity of CO_2 is used in photosynthesis. With sulphide (at a pH of about 8.5; nearly all the sulphide present as NaHS , with some Na_2S !) the "assimilation velocity" amounts to 1230 cmm. per hour. Here, however, only one mol. of CO_2 is *assimilated* when 2 mols of NaHS are dehydrogenated to S, while in the meantime the remaining alkali binds, *purely chemically*, 2 more mols. of CO_2 . Consequently, one should find that per 4H transferred from Na_2SO_3 only 1 mol. of CO_2 is taken up in total, whereas for the same quantity of H transferred from NaHS 3 mols are absorbed. The presence of some Na_2S in the medium will increase this amount somewhat. The actual figures show a ratio of rate of CO_2 absorption of $1230/370 = 3.3/1$, in excellent agreement with the theory.

That CO_2 assimilation with S cannot be measured manometrically as CO_2 uptake in an environment of 0.5% NaHCO_3 results from the production of H_2SO_4 by dehydrogenation of the S. Thus per mol. of S, 6 H atoms are transferred, corresponding to a reduction of about 1.5 mols. of CO_2 . Simultaneously 2 mols. of CO_2 are liberated by the H_2SO_4 from the bicarbonate containing liquid. The net result is, therefore, an increase in total pressure.

Thus these various kinetic studies show at best that CO_2 reduction—not absorption by the (alkaline) medium plus absorption by the bacteria—proceeds at the same rate with different H-donors. And, because in all these experiments the conversion of CO_2 into bacterial substance, and not into a first photosynthate, is determined, they cannot give any indications concerning such a first product.

Gaffron in 1933 (15²) claims to have isolated what he considers to be a direct photosynthate of the formula $(\text{C}_4\text{H}_6\text{O}_2)_n$. Without expressing any doubt that a substance of this kind can be isolated from purple bacteria, it seems to me premature to consider this as the first detectable, or even as an important, intermediate product of photosynthesis by the purple bacteria. The quantity obtained is very small and quite variable, not even varying with the conditions as would be expected. Thus it is stated that the quantity obtained from fresh cultures is considerably smaller than that isolated from old cultures.

In 1935 Gaffron states (15², p. 318): "The fact that substances like acetic acid and lactic acid are reduced in N_2 as well as in H_2 proves that

the main assimilation product of the purple bacteria must possess a higher H-content or a lower O-content than the carbohydrates." This statement should be so understood that the "main assimilation product" ("Hauptassimilationsprodukt") is equivalent to the final product, in other words: the entire bacterial cells.

Thus, with regard to a concept of the mechanism of the photosynthetic conversion of various H-donors and CO_2 into bacterial cells, nothing stands in the way of the hypothesis that fundamentally this conversion follows the same path as green plant photosynthesis.

2. The function of the pigments and the photochemical equivalents.

The fact that photosynthesis requires the absorption of radiant energy implies that the pigments responsible for this absorption must play an important role in this process. Hence an explanation of the photosynthetic reaction must needs involve an explanation of the function of these pigments.

The existence of different types of photosynthesis carried out by different organisms then logically leads one to inquire in how far the essential common features of the various processes can be related to common characteristics of the organisms, and in how far the specific differences can be correlated with differences in the causative agents. Such a comparison may aid in obtaining a deeper insight into the more intimate mechanism of photosynthesis. That in this respect a comparison of the various pigment complexes ranks first is self-evident.

From the foregoing discussion it has appeared that the common feature of photosynthesis in green plants, green bacteria, and purple bacteria is the reduction of CO_2 with the uptake of radiant energy. The available experimental data also indicate that the green pigments, occurring in all three groups of organisms, are mainly, if not entirely, responsible for the conversion of radiant, into chemical, energy. Thus the assumption lies at hand that the photochemical CO_2 -reduction is directly associated with the green pigments.

But the different photosynthetically active organisms require different H-donors for this reduction. Outstanding in this respect are the green bacteria which can utilize only H_2S as such. They are unable to develop either with the other inorganic sulphur compounds or with any one of the organic substances so readily utilizable by the purple bacteria.

This indicates that the green bacteria cannot carry on photosynthesis except in the presence of an H-donor which contains the H in an active form. And it becomes clear that in the purple

bacteria photosynthesis the hydrogen of the other donors must be activated.

Correlated with this difference in available H-donors is the presence of red pigments in the purple bacteria, which are altogether lacking in the green bacteria. This led in 1931 (4) to the hypothesis that these red pigments might function in the activation of the hydrogen in the various donors, and thus play an important part in photosynthesis.

Such a cooperation of the red pigments might require another photochemical process, or proceed in the dark. Experiments carried out in light at a wavelength greater than 6000Å have shown that the conversion of H_2S to H_2SO_4 with the simultaneous reduction of CO_2 proceeds unaltered. In addition, Gaffron has shown in 1934 (13) that purple bacteria photosynthesize actively with $Na_2S_2O_3$ in the infra-red region of the spectrum. These results indicate that a photochemical cooperation of the red pigments is unlikely. Just how they might function in the activation of hydrogen of the donors in the dark could not be decided until more about the chemical nature of these pigments was known.

Meanwhile, another approach to the problem of the function of the pigments was possible. This implies a determination of the quantum efficiency of photosynthesis in different organisms. The fundamental investigations of Warburg and Negelein⁽²¹⁾ have shown that in the photochemical reduction of CO_2 by *Chlorella* four quanta are absorbed per molecule of reduced CO_2 . This indicates the occurrence of four primary photochemical reactions in this process, and many attempts have been made to devise reaction-mechanisms fulfilling this requirement.

The necessity of the absorption of four quanta in green plant photosynthesis follows also from thermodynamical considerations. But the energy relations in purple bacteria photosynthesis are very different. In this process the increase in free energy is so much smaller that one quantum would fulfill the energetic requirements. Thus a determination of the number of quanta involved in the reduction of each molecule of CO_2 in purple bacteria photosynthesis would indicate the number of primary photochemical reactions⁽²²⁾. Such determinations have recently been carried out by Roelofsen. Although the results are still of a somewhat preliminary nature they indicate that per molecule of CO_2 also here 4 quanta are absorbed, and show clearly that one quantum is

entirely insufficient although it would fulfill the thermodynamical requirements.

This points to a very close similarity of the photochemical reactions of photosynthesis in the green plants and the purple bacteria. Further support for this similarity is furnished by recent investigations on the chemical nature of the green pigment of the purple bacteria⁽²²⁾. These studies have shown beyond a doubt that the "bacteriochlorophyll" is chemically closely related to chlorophyll.

On the other hand, recent studies on the chemical nature of the red pigments of purple bacteria have shown conclusively that at least the one pigment isolated so far in sufficient quantity for chemical analysis differs markedly from the corresponding pigments in the green plants⁽²³⁾.

This pigment belongs to the group of carotenoids but is characterized by a higher degree of unsaturation than any one of the representatives of this class so far known.

All these facts make it seem probable that further work along these lines will lead to the accumulation of data of considerable importance for the understanding of the intimate mechanism of photosynthesis.

3. Outlook.

If one summarizes the material discussed in the preceding pages one is led to the conclusion that there is sufficient reason for considering the photosynthetic processes of the green and purple bacteria as reactions very similar to those occurring in green plant photosynthesis. The existing differences in the availability of various H-donors point to a function of the associated red pigments, and it has been set forth that such a function probably does not involve photochemical processes.

The generalized equation for photosynthesis only indicates the ultimate fate of the reacting components, and does not in any way imply a certain mechanism for the transference of H from the various possible H-donors to the one final acceptor, the CO_2 .

The absorption of four quanta for the reduction of one molecule of CO_2 by green plant photosynthesis, where H_2O serves as the final H-donor strongly suggests the activation of four H_2O molecules. This activation obviously would be brought about by the chlorophyll.

²¹ Warburg, O., and Negelein, E., Z. phys. chem., 106, 191, 1923.

²² For a more detailed discussion of this problem see Roelofsen, P. A., Thesis, Utrecht, 1935.

²² Noack, K. und Schneider, E., Naturwissensch. 21, 835, 1933. Schneider, E., Z. physiol. Chem., 226, 221, 1934. Fischer, H., und Hasenkamp, J., Ann. 515, 148, 1935. Clemo, G. R., and McIlwain, H., Chem. and Ind., 54, 135, 1935.

²³ Carnegie Inst. Washington, Yearbook 32, 184, 1933. van Niel, C. B. and Smith, J. H. C., Arch. Mikrobiol., in press.

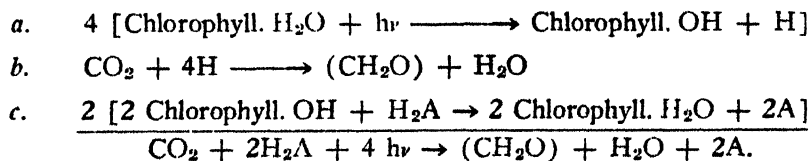
That the same situation apparently is met with in the purple bacteria indicates a similar reaction, the more so on account of the established similarity of chlorophyll and bacteriochlorophyll.

But if the bacteriochlorophyll is capable of activating H in the H_2O molecule, how then are we to understand the necessity of H_2S , H_2 , or organic substances as H-donors, and the complete lack of O_2 -production? At first sight such a scheme would seem to imply that then also in the bacterial photosynthesis H_2O would be the H-donor, O_2 necessarily a product.

If one gives further thought to this problem it appears possible to reconcile the facts with the hypothesis that in all cases of photosynthetic activity one of the reactions would be the activation of H_2O -hydrogen by the green pigment.

The result of such an activation followed by transference of the activated H to CO_2 would be a conversion of the chlorophyll (hydrate) into dehydro-chlorophyll (hydrate). However, in order to keep the process going a reduction of this dehydro-chlorophyll (hydrate) is necessary, and this reduction would be accomplished, in the case of the green and purple bacteria, with hydrogen derived from H_2S , H_2 , or organic substances.

In that event the now familiar generalized equation of photosynthesis becomes the end result of a series of reactions which might be expressed as follows:



Such a series of reactions offers the possibility for a number of different mechanisms for reaction *c*. The simplest case would be a direct reduction of dehydro-chlorophyll (hydrate) by the substance H_2A . It would seem that the green bacteria realize this case, for only the strongly reducing H_2S is here capable of causing the photosynthetic process to proceed. In the purple bacteria one would then be dealing with organisms having at their disposal a mechanism for the transference of H from various substances to the dehydrochlorophyll (hydrate), and the occurrence of a red pigment of a highly unstable nature, lacking in the green bacteria, might offer the explanation for this mechanism.

Whether in the green plants this reduction is accomplished with the participation of the carotenoid pigments cannot as yet be decided. Since here the ultimate dehydrogenation product is O_2 , it would imply the intermediate formation and

decomposition of a substance of a peroxydic nature, and especially since the katalase positive purple bacteria do not produce oxygen, it seems more logical to suppose that the carotenoids are involved rather than the chlorophyll only. The main reason for this is that, as far as the present evidence goes, the chemical similarity between the green pigment in purple bacteria and green plants is much greater than that between the red pigments.

It will be clear that the hypothesis of a photo-decomposition of chlorophyll (hydrate) as the only reaction requiring the supply of radiant energy does not invalidate the generalized reaction, but merely elaborates it. In all those cases where the reduction of the product of this photo-decomposition is brought about by some foreign H-donor, one must look upon this donor as the ultimate source of the hydrogen for CO_2 reduction.

Yet this elaboration brings together many salient facts which heretofore remained obscure. As such I may mention:

1. The necessity of radiant energy.

If the purple bacteria could use the activated hydrogen from organic substances, H_2 , H_2S , etc. directly for the reduction of CO_2 the necessity of light would be hard to understand, unless it were supposed to be necessary for activation of the CO_2 . Yet the methane fermentation offers a

clearcut example of CO_2 reduction in the absence of radiant energy.

2. The necessity of 4 quanta notwithstanding the thermodynamical possibility of photosynthesis in the purple bacteria with only one quantum, if the substance H_2A could furnish the hydrogen for CO_2 reduction without a more intricate mechanism for transference.

3. The similarity of the green pigments in different organisms. This precludes the possibility that activation of the hydrogen of the ultimate H-donor is brought about by a chlorophyll H_2A complex, where H_2A may be H_2S or an organic substance.

4. The nature of the red pigments in the purple bacteria. This also does not justify the assumption that they could form combinations with H_2S or organic substances.

5. The well-known ability of various micro-organisms to catalyze the transference of hydrogen

from H_2S , H_2 , and simple organic substances in the absence of a source of supply of radiant energy.

Although the picture here developed is as yet far from complete, the possibilities of testing various phases by experimental methods is obvious. This consideration has led me to include the remarks made in this last section. They are meant to serve as a working hypothesis which shows on the one hand the importance of a study of the photosynthetic activities of the green and purple bacteria for the general problem of photosynthesis, and on the other hand open the way for devising simple and definite experiments which can bring us closer to an understanding of the intimate mechanism of this process.

DISCUSSION

Dr. Burk: Did you mean to say that carbon dioxide was the only known acceptor of hydrogen which could be supplied externally? Might not other substances, such as nitrate, nitrite, fumarate, or methylene blue function as well?

Dr. van Niel: Gaffron has shown that nitrate is reduced. There may be various other acceptors as well.

Dr. Burk: In regard to different kinds of reducers, have any been found which are inorganic, and do not contain sulfur (apart, of course, from H_2 gas)?

Dr. van Niel: No experiments along this line have been reported so far. But organisms which can carry out photosynthesis with inorganic reducing substances other than sulfur compounds may well exist.

Dr. Burk: Do you mean other than sulfur bacteria, or do you mean these very organisms with which you are working use such compounds?

Dr. van Niel: This question cannot be answered until the experiments have been performed. *A priori* I see no reason why the green and purple bacteria would be the only photosynthetic bacteria.

Dr. Burk: Is it conceivable that the inorganic compounds might be arranged in an oxidation-reduction series, and the various sulfur bacteria arranged in order of limiting potentials?

Dr. van Niel: Certainly.

Dr. Burk: You said the organisms were greatly more reduced than sugar. In the table you gave us, concerning the formic acid experiments, the combined ratio A and B in the last column indicated only a little more reduction, of the order of several percent.

Dr. van Niel: The difference is actually of this order of magnitude.

Dr. Burk: In the autotrophic hydrogen bacteria that reduce CO_2 in the dark the situation is just reversed, the organisms being about 10%

less reduced than sugar (J. phys. Chem., 35, 438, 1931).

Dr. Blum: I am interested in this from the evolutionary standpoint which I think has been disregarded particularly on the basis of energetics. It seems to me difficult to assume that the first photosynthesis, which may have been very different from any of these, involved a four quantum jump. I am interested in what van Niel says, that we have not exhausted the possibilities, because it seems to me that one might expect to find photosynthetic mechanisms involving more elementary steps, unless such organisms have already passed out of existence.

Dr. Burk: I wonder if it is necessary to regard the processes discussed here as requiring a 4 quantum jump. One might consider these reactions as proceeding in 4 consecutive steps, each involving one quantum. It means that nature "decided" to go through the chemical step of reducing water, that it has done so from the start, and that in doing so it has thereby provided a mechanism whereby it can use one quantum at a time.

Dr. Blum: I meant to include the possibility of four consecutive steps, each involving one quantum. This would be difficult to conceive as occurring in one evolutionary step. I can not credit nature with so much foresight as Burk, but think she must have blundered into such a nice mechanism probably by way of several, not so convenient.

Dr. Starkey: Have you been able to use formic acid exclusively in place of CO_2 ?

Dr. van Niel: I have not tried that yet, because the experiments so far have been done under conditions requiring growth. In a medium containing H_2S and formate but no CO_2 growth, although definite, has been too scanty to allow of careful quantitative analysis.

Dr. Starkey: The result of Gaffron that sulphide seems to be oxidized to some extent by the Athiorhodaceae tends to show that these organisms are also facultative autotrophs.

Dr. van Niel: Yet it is impossible to raise Athiorhodaceae in completely inorganic media containing sulphide.

Dr. Starkey: In the case of strictly non-photosynthetic sulfur bacteria the oxidation of the sulfur compound is brought about with oxygen. May it not be that in the photosynthetic forms something is produced within the cell through photosynthesis which is highly oxidized, and with the aid of which the sulfur compounds are oxidized?

Dr. van Niel: I tried to suggest that possibility by reaction C. What the internal mechanism of this reaction actually is, remains as yet unknown.

Dr. Burk: Why put water in the first equation of your scheme, and not just chlorophyll and dehydro-chlorophyll? The equation balances just as well without the H_2O .

Dr. van Niel: Because of the evidence presented by Stoll for the existence of a chlorophyll hydrate, and on account of certain suggestive energetic considerations.

Dr. Inman: What is the most efficient temperature for these organisms?

Dr. van Niel: About $35^{\circ} C$.

Dr. Inman: Would it be your assumption that this type of photosynthesis came before the type we have in the green plant? The higher temperature optimum and the ability to use infrared light might suggest that purple bacteria antedated the green plant as photosynthetic organisms.

What nitrogen compound did you use in your media?

Dr. van Niel: I have used ammonium salts.

Dr. Burk: If you omit ammonia (or other nitrogen compounds) for a long time, do you get any photosynthesis? One would not expect any great difference but curious results of this type are sometimes obtained.

Dr. van Niel: I don't know.

Dr. Inman: Did I understand you to say that any wavelength in the visible or near infrared was sufficient to promote photosynthesis?

Dr. van Niel: No, I meant to say that infrared light between 9200 and 7500 A. where these bacteria have three distinct absorption bands is effective. Further, that red light of a wavelength greater than 6000 A., ordinary incandescent light or sunlight can be used.

CHEMISTRY OF PHOTOSYNTHESIS

N. R. DHAR

Reduction of Carbonic Acid and Formation of Formaldehyde in vitro from Carbonic Acid and Bicarbonates

From the chemical point of view, the main reaction involved in the first stage of photosynthesis seems to be the reduction of carbonic acid to formaldehyde.



Hence, to imitate the main chemical change involved in photosynthesis numerous chemists have attempted to reduce carbonic acid and bicarbonate solutions *in vitro* by different reducing agents. Thus Lieben¹ (1895) and Ballo² (1884) reduced carbonic acid to formic acid by the action of sodium and other amalgams, whilst Moissan (1902) obtained potassium formate by the reduction of carbonic acid by the action of potassium hydride. Fenton³ (1907) by the action of metallic magnesium on carbonic acid obtained formate as the chief product with traces of formaldehyde. On the other hand, Dhar and Atma Ram⁴ (1932) obtained considerable amounts of formaldehyde by the reduction of potassium bicarbonate solutions by powdered metallic magnesium without any trace of formate. This reaction also takes place with carbonic acid instead of bicarbonate. In place of metallic magnesium, cerium, tungsten and iron with carbonic acid have been used with similar results. This reduction of carbonic acid to formaldehyde is accelerated by sunlight. The importance of this research lies in the facts that carbonic acid and bicarbonates are directly converted into formaldehyde, which appears to be also the chief product in the first stage of carbon assimilation, and that the reaction is accelerated by light.

Bredig and Carter⁵ (1914) obtained formic acid by the reduction of carbon dioxide by hydrogen under pressure in the presence of palladium used as a catalyst and some carbonates. Schaper⁶ (1910) reduced carbon dioxide under pressure by ferrous oxalate.

By the action of silent electric discharge on mixtures of carbon dioxide and water, formic acid and formaldehyde were detected along with other products. Fischer and Prziza⁷ (1914) obtained formic acid and traces of methyl alcohol by the electrolytic reduction of carbon dioxide under 10-15 atmospheres. Coehn and collaborators⁸ (1910) observed that dry carbon dioxide is decomposed by extreme ultraviolet light and Berthelot and Gaudechon⁹ (1910) reported that when hydrogen and carbon dioxide are exposed to light, formaldehyde and its condensation products are formed.

Reduction of carbonic acid in the presence of different catalysts has also been effected in the presence of light. Thus Usher and Priestley¹⁰ (1911) and Moore and Webster¹¹ (1913) obtained traces of formaldehyde by exposing carbonic acid and water in the presence of ferric and uranium salts, colloidal ferric hydroxide and some dyes, to ultraviolet and visible light. Stoklasa and Zdobnický¹² (1911-1913) obtained formaldehyde when carbon dioxide and hydrogen with potassium hydroxide were exposed to ultraviolet light. Dhar and Sanyal¹³ (1925) obtained formaldehyde by exposing carbon dioxide to tropical sunlight when it is passed into beakers containing conductivity water. The formation of formaldehyde is facilitated by the presence of methyl orange, methylene blue, ferric chloride, uranyl salt, chromium salt, colloidal ferric hydroxide, chlorophyll, etc. Gopala Rao and Dhar¹⁴ (1931), and Atma Ram and Dhar¹⁵ (1932) obtained formaldehyde by passing carbon dioxide into solutions and suspensions of different substances in water when exposed to tropical sunlight. Nickel carbonate, manganous chloride, and cobalt carbonate produce good results. Methylene blue and malachite green act as good photosensitisers in the formation of formaldehyde from carbon dioxide and water exposed to tropical sunlight.

It is interesting to note that in the presence of several fluorescent substances like rhodamine, fluorescein, cartharamine, safranin, etc., practically no formaldehyde was synthesised photochemically from carbon dioxide and water, although the fluorescent substances were decolourised in the presence of sunlight. Moreover, formaldehyde has also been obtained by exposing solutions of alkali bicarbonates with different photosensitisers. With nascent carbon dioxide obtained by the interaction of hydrochloric acid and carbonate no coloured photosensitisers seem to be necessary for the formation of formaldehyde in tropical sunlight. In all these cases, the yield of formaldehyde was much greater than the limit of sensitiveness of the tests employed. In view of this fact and the careful blank experiments always carried on side by side, there is hardly any doubt that formaldehyde is actually obtained from carbon dioxide and water in tropical sunlight. Moreover, by exposing solutions of potassium bicarbonate alone and with freshly prepared carbonates of zinc, magnesium and iron (ferrous) in sealed glass bulbs appreciable amounts of formaldehyde were obtained.

These researches of Dhar and collaborators have been confirmed by similar observations of Mezzadrolì and his colleagues. Thus Mezzadrolì

and Gardano¹⁶ (1927) have obtained formaldehyde and small amounts of sugar, by exposing solutions of bicarbonates of different metals to ultraviolet light. Ammonium bicarbonate produces a better yield of formaldehyde than the alkali bicarbonates, but the greatest yield of formaldehyde is obtained from calcium bicarbonate. The amount of formaldehyde generated rises to a maximum and then gradually decreases owing to its oxidation and polymerisation. Moreover, Mezzadroli and Vareton¹⁷ (1928) have shown that exposure of these bicarbonate solutions and carbonic acid to ultraviolet rays causes an increase in the reducing powers of the solution to a maximum followed by a rapid fall. The presence of colloidal or reducing catalysts increases the reducing powers. Mezzadroli and Vareton have also reported that the yield of formaldehyde and sugars is increased by the addition of metallic magnesium to the calcium bicarbonate solution. Mezzadroli and Babes¹⁸ (1929) have stated that the reducing power of a 5% solution of potassium bicarbonate exposed to ultraviolet light increases to a constant value in the presence of an active variety of carbon. When zinc is present with the carbon the reducing power is still further increased.

Recent experiments carried on in these laboratories with great care along with blank experiments, show that formaldehyde is synthesised and detected when dilute solutions (5%) of bicarbonates of the alkali metals are exposed to sunlight for about 4 hours in thin layers (0.5 cm. thick) either in open dishes (11 cm diameter) or in dishes covered with silica plates at temperatures up to 30°. Higher temperatures are prejudicial to formaldehyde production. The amount of formaldehyde photosynthesised per 100 c.c. of solution exposed is 0.00007-0.0001 g. Schryver's reagent is most sensitive for the detection of formaldehyde in small quantities. The amount of formaldehyde obtained from exposing the bicarbonate solutions in the same dishes placed in a bath at 40° is about one third of that obtained at 30° under identical conditions.

It will be of interest to note that in nature the amount of carbon assimilation is less at 40° than at 30° as will be evident from the following observations.

Miss G. L. C. Matthaei¹⁹ (1905) observed that the amounts of CO₂ assimilated by a cherry laurel leaf per 30 sq. cm. per hour at various temperatures were:

Temperature	6°	8.8°	11.4°	15°	23.7°	30.5°	37.5°	40.5°	43°
Weight of CO ₂ assimilated (g.)	0.0002	0.0038	0.0048	0.0102	0.0070	0.0157	0.0238	0.0149	0.0102

But the total dry weight of organic matter produced during the whole life of the plant may not increase with temperature in this way. Bialoblocki's (1871) results with barley are as follows:

Temperature	0°	10°	20°	30°	40°
Dry matter formed	0	7.64	8.22	3.85	0.93

Similar results have also been obtained by Baly and collaborators *in vitro*.

In tropical countries, the optimum temperature of photosynthesis in plants, as a rule, seems to be higher than with plants growing in temperate climates.

Since 1870 when Baeyer gave out his formaldehyde hypothesis, numerous attempts have been made to obtain formaldehyde *in vitro* from carbon dioxide and water on exposure to light. Usher and Priestley (1911), Baly, Heilbron and Barker²⁰ (1921), Dhar and co-workers (1925-32), Mezzadroli and collaborators (1927-29), Yoe and Wingard (J. Chem. Phys., 1933 1, 886) and others obtained positive evidence of formaldehyde formation from carbonic acid or bicarbonates in the presence of catalysts, when exposed to light. On the other hand, Spoehr²¹ (1923), Baur and Rebmann²² (1922), Porter and Ramsperger²³ (1925), Bell²⁴ (1931), Emerson²⁵ (1929), Zscheile²⁶ (1932), Mackinney²⁷ (1932), and Qureshi and Mohammad²⁸ (1932) obtained negative results although Mackinney made the following significant statement:

"The status of this problem is extraordinarily involved, though it can hardly be doubted that some workers have succeeded in obtaining formaldehyde *in vitro*."

Baly and co-workers²⁹ (1927) seem to contradict their earlier results.

Formaldehyde in Rain Water and in Upper Atmosphere

Recently a new aspect of the problem has been brought forward by the observations of Dhar and Atma Ram³⁰ (1932-1933), that freshly collected rain water always contains appreciable amounts of formaldehyde. It is believed that formaldehyde in rain water is formed by the combination of carbon dioxide and water vapour present in the atmosphere by the absorption of ultraviolet light from the sun.

We have continued our analyses of rain water for formaldehyde and we have observed that all samples of rain water contain formaldehyde varying from 0.00015 to 0.0012 g. per litre. It is inter-

esting to note that the amount of formaldehyde per litre of rain water obtained after some sunny days is practically the same as that photosynthesised by exposing solutions of potassium bicarbonate to sunlight. It may be that the amount of formaldehyde in both cases is controlled by the equilibrium,



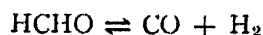
We have shown that the incidence of lightning discharge and thunderstorm does not increase the amount of formaldehyde present in rain water. On the other hand, we have observed that the amount of formaldehyde present in rain water is greater when the rainfall is preceded by some clear sunny days. Hence we are inclined to the view that formaldehyde in rain water is obtained as a result of its photoformation from carbon dioxide and water in the atmosphere.

It is well known that the following reaction requires ultraviolet light of wavelength 2550Å. $\text{CO}_2 + \text{H}_2\text{O} + 112,000 \text{ cal.} \rightarrow \text{HCHO} + \text{O}_2$. It is apparent that very seldom all the active rays are absorbed by a medium and hence it seems that all short ultraviolet rays coming from the sun will not be absorbed by the ozone layer present in the atmosphere. Some of the short wave radiations are likely to pass through the ozone layer and decompose water into H and OH, and these hydrogen atoms may reduce CO_2 to formaldehyde as has been observed by P. Hardeck³¹ (1933). This reduction of carbon dioxide by atomic hydrogen may be accelerated by the solar radiations. The heat of dissociation of water into H and OH is 110,000 calories. In other words, the energy required in the formation of formaldehyde from carbon dioxide and water is practically the same as that required in the breaking of the H—OH link, which appears to be the first step in this process.

As the wavelength necessary for the formation of ozone (2020Å) is shorter than that necessary for the formation of formaldehyde (2550Å), it is expected that formaldehyde may be formed in the atmosphere at a height less than that of ozone.

Henri and Schou³² (1928) and Herzberg³³ (1931) have shown that the ultraviolet absorption spectrum of formaldehyde vapour consists of 35 to 40 bands between 2500 and 3700Å with a maximum at 2935Å characteristic of aldehydes. The predissociation limit of formaldehyde appears to lie between 2680 and 2660Å with diffuse bands. On irradiating formaldehyde vapour with rays of wavelength between 2800 and 2650Å, Kirkbride and Norrish³⁴ (1931) obtained a quantitative decomposition of formaldehyde into CO and H_2 .

It is apparent, therefore, that not only ozone but also formaldehyde present in the atmosphere absorbs short rays from the sun. Hence the absorption of solar radiations shorter than 2900Å, which has been so far attributed to the presence of ozone, may be partially due to the formaldehyde present in the atmosphere. Just as there is an equilibrium in the atmosphere between the oxygen and the ozone, it is evident that the following equilibrium may exist in the atmosphere.



It is well known that the upper atmosphere is rich in hydrogen. Consequently, due to the presence of hydrogen, the photodecomposition of formaldehyde will be markedly hindered and appreciable amounts of formaldehyde may exist in the atmosphere.

From the foregoing lines it will be seen that the wavelengths of radiations suitable for formaldehyde formation (2550Å) and decomposition (2660Å) are much nearer each other than in the formation (2020Å) and decomposition (2655Å) of ozone. Hence, there is greater likelihood of the decomposition of the formaldehyde as soon as it is synthesised than in the case of ozone, but due to the presence of hydrogen, an appreciable amount of formaldehyde is likely to exist in the atmosphere.

Just as ozone can exist in the atmosphere at a height of a few kilometers above the earth's surface (compare Gotz, Dobson and Meetham, *Nature*, 1933, 132, 281) formaldehyde can also exist at similar heights and that is why it can be washed down by rain water, which has been found to contain formaldehyde. We have made many careful experiments to see whether air on the surface of the earth contains appreciable amounts of formaldehyde. Large volumes of air were aspirated slowly through 20 c.c. of distilled water contained in a glass tube from 6-24 hours, but no trace of formaldehyde could be detected in the water by applying the Schryver test, showing thereby that although formaldehyde exists in the upper atmosphere, it does not occur appreciably in the atmosphere near the surface of the earth.

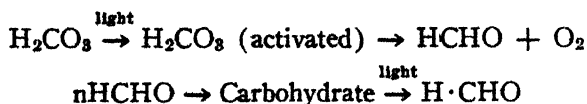
Formation of Sugars from Formaldehyde

Thanks to the researches of several chemists, the problem of the formation of reducing sugars by the condensation of formaldehyde is on a better footing than that of the photosynthesis of formaldehyde from carbon dioxide and water vapour. Butlerow (1861), Loew (1886-1889), Fischer (1892-1905), von Euler (1906), Nef (1914), Spöhr³⁵ (1926) and others have shown that under various conditions, especially in the

presence of alkalis, formaldehyde is converted into reducing sugars in the absence of light.

Spoehr obtained traces of sugar by exposing 3% solutions of formaldehyde with zinc carbonate or potassium nitrate to sunlight. With lead and calcium hydroxides, which form sugars in the dark also from formaldehyde, the yield in light was greater. Inghilleri (1912) obtained sorbose by exposing formaldehyde and oxalic acid to light, whilst Pribram and Franke (1912) prepared glycollic aldehyde by exposing formaldehyde to ultraviolet light in quartz vessels.

Baly and collaborators⁸⁶ (1924-1931) have carried on important researches on the conversion of formaldehyde to reducing sugars in the presence of ultraviolet light. Baly (1924) reported that "with an initial concentration of 40% formaldehyde, the maximum reducing power is 8% calculated as glucose and with 20 litres of formaldehyde, this can often be reached after 14 days of continuous illumination." Recently Baly and collaborators (1927) have denied the formation of formaldehyde from carbon dioxide and water in ultraviolet light as reported in their previous work, but have obtained glycol, glycerol and reducing sugars from exposing formaldehyde to ultraviolet light. They have shown that when a 40% formalin solution containing an excess of calcium carbonate is placed in a tank kept at 30° and exposed to ultraviolet light from four quartz mercury vapour lamps for a month and the mixture is stirred, 80% calcium formate, 5.6% calcium glycolate and 15% of a mixture containing glycol, glycerol, pentaerythritol and some reducing sugars are obtained. According to these authors, the action of ultraviolet light is represented by the following scheme:



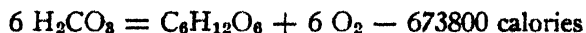
It is believed that the following stationary state is established by the action of ultraviolet light on carbonic acid:



When the concentration of carbohydrate is small and in the presence of reducing agents, the reaction would proceed from left to right with the formation of carbohydrates, which would be photochemically decomposed to formaldehyde. Moreover, Baly and co-workers⁸⁷ (1931) observed that when various sparingly soluble substances, capable of absorbing carbon dioxide were used and carbon dioxide was passed and the whole was exposed to ultraviolet light, complex organic compounds containing carbohydrates, which char readily and develop reducing power after hydrolysis with hydrochloric acid, are

formed. Among the powders which behave in this way were metallic aluminum, barium sulphate, freshly precipitated aluminum hydroxide, basic carbonates of magnesium and zinc, ferric, chromic, and aluminum hydroxides with small amounts of thorium hydroxide deposited on kieselguhr, etc. They also used colored substances like nickel or cobalt carbonate alone or deposited on kieselguhr with small amount of thorium carbonate in aqueous carbonic acid and visible light. The organic substances formed reduced Benedict's solution, gave the Rubner and Molisch reactions and formed a solid osazone. Under comparable conditions the use of visible light and coloured surfaces gave a greater yield of organic matter with a higher carbohydrate content than the use of ultraviolet light and white surfaces. It appears that the exclusion of ultraviolet light prevents the photodecomposition of the carbohydrates formed. When a solution of ammonium carbonate containing a suspension of nickel or cobalt carbonate is exposed to visible light, complex nitrogenous organic compounds are formed.

Baly and collaborators have pointed out that the thermochemical equation,



requires the wavelength 2552Å for the activation of carbonic acid by means of radiation alone. Since photosynthesis occurs in the plant with visible light, some other mode of activation must be discovered. These authors state "the total quantity of energy necessary for photosynthesis to take place is supplied in two separate amounts, one quantity being given when the adsorption on the surface takes place, and the second quantity being given out by light." It should be pointed out that a similar behaviour is observed with several other photochemical reactions, which are sensitised by different substances.

Recently Baly and Hood (1929) have shown that if the yield of carbohydrates (weight of photosynthesised organic matter soluble in absolute methyl alcohol) obtained from the presence of a specially purified suspension of nickel carbonate (50 g.) in 1000 cc. of water, is plotted against the temperature, the relation is found to be a linear one between 5° and 31° (maximum yield being 0.0783 g.); after which there is a rapid decrease of yield. The values of the temperature coefficient for a 10° rise are in good agreement with those observed by Warburg (1919) with the unicellular alga, *Chlorella*, under constant illumination. Baly and Hood have pointed out the close analogy between the photosynthesis *in vitro* and *in vivo* with special reference to the researches of Miss Matthaei (1905) on the assimilation of carbon dioxide at various temperatures and the fact that the process both in the living

leaf and in the laboratory has an upper and a lower temperature limit.

Dhar and co-workers²⁸ (1925-1932) have studied the conversion of formaldehyde solutions to reducing sugars in the presence of catalysts in sunlight. Dhar and Sanyal (1925) and Atma Ram and Dhar (1932) obtained reducing sugars by exposing solutions of formaldehyde with ferric chloride to sunlight. When solutions of formaldehyde are exposed to sunlight for periods varying from 60 to 125 hours with catalysts like ferric chloride, zinc oxide, nickel carbonate, chlorophyll, methylene blue and methyl orange, reducing sugars are detected. The best results obtained so far are those with ferric chloride. It will be of interest to note that formaldehyde solutions when mixed with fluorescent substances like safranine, cartharamine, rhodamine, etc., and exposed to sunlight, do not form reducing sugars, whilst with chlorophyll, reducing sugars are produced. The sugars obtained reduced Benedict's solution and gave the Molisch and Rubner reactions. On exposing 1% solution of formaldehyde in the presence of freshly prepared dilute ferric chloride in thin layers in open dishes, reducing sugars can be obtained even after an exposure of 4 hours to sunlight.

Influence of Temperature on Sugar Formation from Formaldehyde

As the yield of reducing sugars on exposing formaldehyde solutions to sunlight was best with ferric chloride it was thought profitable to determine the temperature coefficient of this polymerisation for a 10° rise of temperature. 800 cc. of 3% formaldehyde solutions were exposed in a sealed bulb with ferric chloride to sunlight for 45 hours at 30° and 40°. After the removal of the ferric and ferrous salts formed by the reduction of the ferric chloride, the solutions were evaporated to complete dryness, and freed from formaldehyde. The dried mass was extracted with pure methyl alcohol. The residue obtained after removal of methyl alcohol was estimated by the reduction of Fehling's solution. The amount of CuO obtained at 30° = 0.061 g. \equiv 0.1355 g. of glucose, whilst at 40° the CuO = 0.077 g. \equiv 0.16 g. of glucose.

Hence the temperature coefficient for a 10° rise of temperature between 30° and 40° for the conversion of formaldehyde solutions to reducing sugars in the presence of ferric chloride in sunlight is 1.2. In this connection, it will be of interest to note that van Amstel (1916) obtained the value 1.25 for 10° rise of temperature between 24° and 36.5° in photosynthesis with *Elodea* and Sir J. C. Bose²⁹ (1924) obtained the value 1.22 for a 10° rise between 20° and 30° in photosynthesis with *Hydrilla*.

Photosynthesis of Nitrogenous Compounds.

From our experiments on exposing solutions of formaldehyde and nitrate or ammonia to sunlight, we observe that methylamine is fairly easily obtained. Hence, it appears that in the absence of carbohydrates, the products of photosynthesis of nitrogenous compounds will essentially consist of substances like pyridine, piperidine, etc., formed by the reaction of methylamine and formaldehyde, and these compounds have actually been obtained by several workers including ourselves. In the presence of carbohydrates, however, we are likely to obtain alkaloids like nicotine, but especially amino-acids by the reaction of aldehydes of the monobasic and dibasic acids obtained from starch and carbohydrates with ammonia or methylamine. It appears, therefore, that in nature also, amino-acids, the precursors of proteins, are formed by the reactions of ammonia or methylamine on the derivatives of carbohydrates and that is why the formation of proteins in plants goes hand in hand with the formation of carbohydrates, which require light. Several workers have reported that the formation of protein in nature is facilitated by the presence of carbohydrates or light. Moreover, it appears that protein formation in plants is likely to be facilitated by the presence of fats, which yield glycerol readily. It has been shown by Dhar and collaborators that reducing sugars are obtained by exposing glycerol to sunlight. Recently we have observed that reducing sugars are obtained by exposing, to air and to light, solutions of tartaric acid and other organic acids in the presence and absence of photocatalysts. Hence the presence of tartaric acid or other hydroxy organic acids may also favour the formation of proteins in plants.

It is well known that in the animal body, proteins are converted into glucose. In the plant kingdom the formation of proteins is facilitated by the presence of glucose. It appears, therefore, that in photosynthesis protein formation is likely to take place when some carbohydrates have already been formed.

Appreciable amounts of nicotine have been photosynthesised and the molecular weight of the base determined from the chloroplatinate of the base on exposing solutions of ammonia, formaldehyde and cupric salts in the presence of catalytic surfaces like ZnO, TiO₂, etc., to sunlight for about 80 hours. Moreover, when solutions of glycol and potassium nitrate are exposed to sunlight for about 8 hours in the presence of TiO₂ as a photocatalyst, tests for glycine are obtained. Similarly a solution containing glucose and potassium nitrate with TiO₂ as a photocatalyst when exposed to sunlight for the same period, appears to produce arginine. Longer exposure causes the disappearance of the amino-

acids photosynthesised, probably due to their photo-oxidation. Solutions of ammonium lactate form amino-acids when exposed to light. These amino-acids obtained in photosynthesis can be readily tested by the valuable "ninhydrin" test. (Compare Dhar and Mukherji, J. Indian. Chem. Soc., 1934, 11, 727).

Influence of Temperature on Photosynthesis.

Many plant physiologists following the lead of Blackman have applied the van't Hoff rule to plant temperature studies. The application of the Arrhenius relation has been found to be general with ordinary chemical reactions. When the same relation is applied to the results actually obtained regarding the influence of temperature on photosynthesis in plants, it fails, as will be evident from the following table obtained from Warburg's⁴⁰ results (1919).

Light Intensity	Observed temperature coefficient ($kt + 10$)/ kt	Calculated temperature coefficient ($kt + 10$)/ kt
16	2.0	4.11 between 16° and 25° (taking 4.7 between 5° and 10°)
45	2.0	4.01 between 10° and 20° (taking 4.3 between 5° and 10°)
45	1.6	3.66 between 20° and 30° (taking 4.3 between 5.4° and 10°)

These results have been calculated by applying the well known Arrhenius relation: $\log (k_1/k_2) = [A(T_1 - T_2)]/T_1 T_2$. It appears that the temperature coefficients of photosynthesis do not obey the Arrhenius relation which has been found to be universally applicable to ordinary chemical reactions investigated so far and no case of failure has been reported. In photosynthesis, the observed values are always smaller than the calculated ones. The reasons for the non-applicability of this relation to photosynthesis in plants are: (1) the greater influence of temperature on the respiration process than that on photosynthesis and (2) the harmful influence of high temperature on the chloroplast.

When the temperature of a plant system undergoing photosynthesis is increased the velocity of photosynthesis is increased but to a smaller extent than that of respiration. Consequently, the temperature coefficient of the observed photosynthesis will appear to be smaller than when the reversible reaction is not present. Moreover, the chloroplast in the protoplasmic cell which is likely to be active in the photosynthetic process starts undergoing deterioration

when the temperature is greater than 20° and may be partially destroyed when the temperature is still greater. This is evident on comparing the results obtained by Warburg and those calculated from the Arrhenius relation. The observed temperature coefficients between 16° and 25° and between 10° and 20° are nearly half of the calculated values, whilst the observed temperature coefficient between 20° and 30° is much less than half of the calculated value. The pernicious influence of high temperature on physiological and enzymatic and bacterial processes is well known. In most cases the optimum temperature in these reactions is around about 20°. Moreover in plant photosynthesis, there is an additional factor, namely, the reverse reaction, i. e., respiration, which is also simultaneously going on and is counterbalancing the photosynthetic reaction and hence, the influence of temperature on photosynthesis is less pronounced due to these counteracting agencies.

It has been observed that in the case of some chemical reactions, the temperature coefficient can have the high value 7.2. Hence it is no wonder that the temperature coefficient of photosynthesis at low temperatures (say between 5° and 10°) has the value 4.3. It seems probable that the photosynthetic reaction is not an adsorption process of which the average temperature coefficient is in the neighbourhood of 1.2 for a ten degree rise of temperature, but it is controlled by a truly photochemical change having a moderately high temperature coefficient. In several communications from our laboratories it has been shown that the photochemical reactions need not have temperature coefficients approaching unity, but can have values as high as 4. (Compare Dhar "Chemical Action of Light," 1931, pp. 314-318). From the foregoing considerations, it is clear that it is needless to assume that the photosynthetic process involves two reactions. It is believed that in high light intensity the chemical reaction ("Blackman reaction" as designated by Warburg) is determining the total velocity of the reaction, because for a ten degree rise of temperature between 15° and 25° the velocity of the photosynthesis is doubled. On the other hand, in low light intensity, the temperature coefficient instead of being 2 as with intense light, is 1.06 and hence it has been assumed that the chemical reaction is not the controlling factor as in the previous case, but the photochemical reaction with a low temperature coefficient determines the photosynthetic rate at low intensities of light.

In the presence of intense light, the photochemical reaction causing the photosynthesis and having a moderately large temperature coefficient is predominant and the counteracting influence of the respiration process, which is not as much accelerated by light as the photosynthetic reaction,

is not prominent. On the other hand, in the presence of feeble illumination, the velocity of the photosynthetic reaction is not high, because this reaction takes place only in light and is proportional to the light intensity. In this case, the counteracting influence of respiration, especially at increased temperatures, becomes prominent and hence the influence of temperature on the observed photosynthetic rate is feeble.

Warburg (1919) has observed that the temperature coefficient of photosynthesis with the unicellular alga *Chlorella* is much less when the light intensity is feeble than when it is strong. Thus $kt + 10/kt$ between 16° and 25° with light intensity sixteen = 2.0, and $kt + 10/kt$ between 15° and 25° with a relative intensity of one = 1.06.

These results which appear to have been confirmed by other workers can be explained in the following way. It has already been stated that in a plant, the following opposing reactions are taking place: $n\text{CO}_2 + n\text{H}_2\text{O} \rightleftharpoons \text{C}_n\text{H}_{2n}\text{O}_n + n\text{O}_2$; and the temperature coefficient of photosynthesis is less than that of respiration. Hence, when the light intensity is feeble, the velocity of photosynthesis is small and is slightly greater than that of respiration at the same temperature. Now when the temperature of the system is raised through 10 degrees, the velocity of the photosynthesis will be increased to a smaller extent than that of respiration. Consequently the temperature coefficient of the observed photosynthesis may be unity or less.

Moreover, in nature when the temperature of the air is high, the plants gain no material through photosynthesis because of the high respiration, whilst at lower temperature with the same light intensity, food materials are formed in the plant.

Willstätter and Stoll⁴¹ (1918) have reported that leaves of low chlorophyll content exhibit a lower acceleration with increasing temperature than the leaves of high chlorophyll content. Thus leaves of *Ulmus* with low chlorophyll content showed a temperature coefficient of 1.34 and with high chlorophyll content of 1.53 between 15° and 25°. These results of Willstätter and Stoll can be explained from the view-point already advanced.

The temperature coefficient (1.53) of the photosynthesis with chlorophyll-rich leaves is greater than that with chlorophyll-poor leaves (1.34), although the photosynthesis is not at all directly proportional to the amount of chlorophyll in the leaves. Willstätter and Stoll find that temperature variations do not affect the rate of photosynthesis of the yellow varieties as much as the normal ones. In the yellow varieties, the amount of photosynthesis being small, the compensating influence of respiration becomes promi-

nent and hence temperature does not appear to influence photosynthesis with these varieties to the same extent as the normal ones with more chlorophyll.

Moreover, differences in light intensity have more profound effect on the yellow varieties than on the normal ones and the time factor appears more slowly than with the normal ones. It is well known that photosynthesis increases with the light intensity and the chlorophyll content of the leaves. Now in the case of leaves containing much chlorophyll, the velocity of photosynthesis will be high and may reach the maximum, even when the light intensity is not high and hence in these cases, the reaction will be less sensitive to the influence of light changes, because the reaction is already fast, due to the presence of large amounts of chlorophyll. On the other hand, when the chlorophyll content is small, the reaction velocity is small and light will affect the velocity more markedly than in the previous case. This explanation is in agreement with the observations of Willstätter and Stoll that in the chlorophyll-rich leaves, an increase of light intensity was without influence on photosynthesis; in fact the light intensity could be reduced by $\frac{3}{8}$ without affecting the rate of photosynthesis. Exactly similar exhaustion effect has been observed with several photochemical reactions where the velocity of the reaction may be proportional to $I^{1/2}$ or $I^{1/4}$ in some cases where the reaction is very fast. (I = light intensity) (compare Bhattacharya and Dhar, J. Indian. Chem. Soc., 1929, 6, 197, 523).

The Phenomenon of "Solarisation"

It is well known that not only high temperature but also long exposure to strong light affects photosynthetic activity. Thus Ursprung⁴² (1917) observed that a leaf of *Phaseolus* after 5 hours of illumination showed very deep coloration of the starch-iodine, while after 8.5 hours' illumination, the reaction was faint. This phenomenon can be observed with almost any source of light of sufficient intensity and the time required is proportional to the light intensity. The effect is first brought about in the red orange portion, the region showing the best photosynthetic activity. With higher intensity, the shorter wavelengths bring the effect about in shorter time and it is apparently proportional to the photosynthetic activity of light. Ursprung has called this phenomenon "solarisation" as it is analogous to the phenomenon of solarisation observed in photographic plates under similar circumstances.

It is expected that not only with starch but with other carbohydrates, a similar effect will be observed. This behaviour has been ascribed to the inactivation of chloroplasts. After long exposure to intense light, the plant organs are assumed not to function, although they are not

killed and on keeping in the dark for a period, again produce starch normally.

The inhibiting effect of long exposure to light of high intensity on photosynthesis has been studied by Ewart⁴⁸ (1897) and the inhibiting effect has been ascribed to the destruction of chlorophyll. Pantanelli⁴⁴ (1903) explains the fatigue effects observed by him in bright light from the view-points of chlorophyll destruction and injury to the chloroplast plasma. The observations of Ewart on *Allium cepa*, which does not form starch, indicate that when leaves of this plant are exposed to bright light for 14 days or for a shorter period while being fed with sugar, the evolution of oxygen finally ceases. This inactivation apparently does not injure the cells or chloroplasts. After a few days in darkness, the capacity for photosynthesis is regained.

The foregoing facts are explained from the following considerations.

In plants the following equilibrium exists:



The direct action (photosynthesis) is being opposed by the reverse reaction (respiration), which will increase, according to the law of mass action, with increase in the concentration of the carbohydrate, which is a product of photosynthesis. Consequently, with accumulation of carbohydrates or when the plants are fed with sugar, as was done by Ewart, photosynthesis is retarded and may stop altogether when the carbohydrate content becomes very high. When the illumination is high and it lasts for a long time, the carbohydrate content increases and along with it the respiration also increases, and thus the photosynthetic velocity falls off with time even when the illumination is continued. After a time the respiration will more than counterbalance photosynthesis and the carbohydrates formed by the photosynthesis will be oxidised to carbon dioxide and water and will disappear on prolonged exposure. When the carbohydrates disappear, the photosynthesis will again begin. It has been known for a long time that the photosynthetic rate decreases with accumulation of the products of photosynthesis. Moreover, Saposchnikoff⁴⁵ (1893) has demonstrated the inhibitory power of an accumulation of carbohydrates and that these cannot increase beyond a certain point. When the leaves of *Vitis vinifera* contain 23 to 29% carbohydrates in dry weight, photosynthesis ceases and respiration predominates. Saposchnikoff has shown that as carbohydrates accumulate, decrease of photosynthetic rate takes place, whilst a decrease in the carbohydrate content results in an increased photosynthesis. These results are evident from the view-point of the reversible reactions already put forward.

Moreover, there are two other factors which

increase respiration, which should be considered, viz., (1) influence of light intensity on the respiratory process, and (2) influence of increased temperature caused by prolonged light absorption.

Compensation Point

The compensation point, i.e. the light intensity at which the photosynthetic and respiratory activities of the plant compensate each other, decreases with decrease of temperature as will be evident from the following table:

Plant	Light Intensity at 20°	Light Intensity at 5°
<i>Spirogyra</i>	174	26.7
<i>Fontinalis</i>	150	40.
<i>Cladophora</i>	253.3	62.9
<i>Cinclidotus</i>	400	75.

The foregoing results show that the light intensity which at 20° represented the compensation point produced an evolution of oxygen due to photosynthesis at 5°.

With *Cladophora*, with increasing temperature, the compensation point rises more rapidly than the rate of respiration determined in the dark; an increase of temperature from 5° to 25° causes the respiration to become 4.8 times greater in the dark, whilst the light intensity increases 6.69 times.

The foregoing results as well as other facts regarding the compensation point can be explained from the following considerations:

1. Photosynthesis is proportional to the light intensity, there being no photosynthesis in the dark.
2. Respiration takes place in the dark but is appreciably accelerated by light.
3. An increase of temperature affects respiration more markedly than photosynthesis.

The fact that the compensation point rises with increase of temperature is due to the greater increase of respiratory activity than photosynthetic activity with increased temperature. The respiratory activity of the plant, which counterbalances the photosynthetic process, increases much more than photosynthesis at higher temperatures and consequently, the light intensity must be increased to cause more photosynthesis to counteract the increased respiratory activity. There is another reason for further increase in the respiratory activity of the plant. Hitherto, it has been assumed by most of the plant physiologists that the process of respiration is not accelerated by light. But it is evident from the researches of Dhar and collaborators (vide, "New Conceptions on Biochemistry," 1932) that animal metabolism is markedly accelerated by light absorption. Hence, it seems pretty certain that the respiratory process taking place in plants is also accelerated by light.

Consequently the respiratory activity of the plant is accelerated by two agencies, temperature and light intensity, and thus the light intensity required for increased photosynthesis in order to counteract this high respiratory activity should be very high. Thus with increasing temperature, the compensation point should rise more rapidly than the rate of respiration because of its additional enhancement by light absorption and this is clearly borne out from the experiments on *Cladophora* in which an increase of temperature from 5° to 25° causes the respiration to become 4.8 times greater when determined in the dark, whilst the light intensity increases 6.69 times, for the compensation point.

It is evident that under certain circumstances, when the temperature is high and the light is intense, the compensation point may not be attained even with intense light and the plant will evolve carbon dioxide like an animal even in presence

plant with reference to temperature is naturally of great importance to the life of the plant and its relation to the environment.

Formaldehyde a General Product in the Photo-oxidation of Organic Compounds

When solutions of organic substances like acetic acid, citric acid, glycine, malic acid, lactic acid, glycogen, acetone, etc., are exposed to sunlight and air, formaldehyde is readily obtained. Dyes like malachite green, methyl violet, methylene blue, etc., also form formaldehyde readily on photo-oxidation. Tartaric acid, butyric acid, propionic acid and some dyes form smaller quantities, whilst oxalic acid, formic acid, glucose, cane sugar, starch, histidine, etc., produce very small amounts of formaldehyde from photo-oxidation. The following are some of the results obtained by us—

Comparative Experiments in the Photosynthesis of Formaldehyde *in vitro* from Sodium Salts of Fatty Acids, Carbohydrates, Proteins and Potassium Carbonate Solutions Exposed to Sunlight in Quartz Vessels.

Temperature 35°, Volume of Solution Exposed = 25 c.c. for 6 Hours.

System exposed	Amount of substance decomposed or photo-oxidised in gram moles	Percentage of the substance oxidised or decomposed	Amount of formaldehyde formed in gram moles	Ratio of amount of substance oxidised or decomposed to that of formaldehyde formed
M/5 KHCO_3	0.098	4.9	0.000001	9800
M/100 Sodium Oleate	0.00011	1.1	0.0000037	29
M/100 Sodium Palmitate	0.000091	0.9	0.000003	30
M/100 Sodium Stearate	0.000082	0.82	0.0000023	36
M/100 Cane Sugar	0.00015	1.5	0.0000018	83
M/100 Glucose	0.00016	1.6	0.0000017	126
M/100 α -Alanine	0.00021	2.1	0.0000018	117
M/100 Aspartic Acid	0.00018	1.8	0.0000015	120

of light. This is likely to happen frequently in tropical countries where at the sea level, the heat rays of the sun become very prominent and the temperature of the plant will be high and photosynthesis cannot counterbalance respiration under these circumstances. At higher altitudes, the light rays are more active than at the sea level and it is expected that at these altitudes, respiration will very seldom exceed photosynthesis in sunlight.

These conclusions are corroborated from the experimental results of Harder⁴⁶ (1921) with sea plants in the polar zones where the light intensity is not very high. Thus Harder records the following ratio of photosynthesis and respiration for different temperatures:

20°—22°	→ 0.588	0.4427	0.4280
2°—3.5°	→ 1.603	0.9207	2.059

The position of the compensation point of a

The foregoing results show that the amount of formaldehyde formed by exposing potassium bicarbonate solutions to sunlight in quartz vessels is smaller than the amount formed in the photo-oxidation of the organic substances. Moreover, when we compare the amounts of formaldehyde formed with the number of molecules of bicarbonate decomposed or the organic substance oxidised, a great difference is at once observed. Although the salts of the fatty acids are oxidised to a smaller extent than the carbohydrates and the amino acids under comparable conditions, the amounts of formaldehyde produced are greater in the case of the salts of the fatty acids than with carbohydrates and amino acids.

It is well known that the amount of heat generated per gram of fat oxidised is 9 calories whilst with both carbohydrates and proteins it is 4.1 calories. It seems, therefore, that the amount of formaldehyde formed during these photo-oxi-

dations increases with the quantity of energy liberated in the photo-oxidations of the organic compounds.

It seems likely that the energy generated in the photo-oxidation of these organic substances supplies a part of the energy for the photoformation of formaldehyde. We are of the opinion that in nature, the photosynthesis that is taking place in the plants is aided by the energy obtained in plant respiration, which goes on as long as the plant lives. The ease with which formaldehyde or other energy-rich compounds are formed in plants is partly due to their getting a constant supply of energy from the oxidation of the food materials present in the plant. We have postulated that the most important chemical change in the formation of carbohydrates in plants and in the formation of formaldehyde in nature from carbon dioxide and water is the photolysis of water into H and OH. The amount of energy required to decompose a gram molecule of water into H and OH is approximately the same as that necessary for the formation of a gram mole of formaldehyde from carbon dioxide and water. These are highly endothermal changes requiring radiations of wavelength 2550\AA (112,000 calories). In nature, however, photosynthesis takes place in visible light, especially the red. We are of the opinion that the energy derived from respiration in the plants already supplies a part of the energy necessary for the photosynthesis and thus renders the photodecomposition of water possible by longer wavelengths. Although the adsorption of carbon dioxide and water by the chlorophyll of the leaf may partially activate these substances just as the adsorption of hydrogen and oxygen on a platinum or palladium surface renders them active, it appears to us that this activation of carbon dioxide and water by their adsorption on the leaf surface is less important than their activation by the absorption of energy from respiration.

There is an intimate relation between respiration and photosynthesis in the plant kingdom, because photosynthesis cannot proceed without the energy available from respiration for the partial activation of carbon dioxide and water vapour. The need of the presence of oxygen in photosynthesis is also explained from the same point of view.

It is easier to obtain formaldehyde or any other energy-rich compound from carbonic acid or bicarbonate solution on exposure to light when a suitable exothermal reaction is taking place in the system along with the photosynthetic reaction. Dew has been found to contain appreciable amounts of formaldehyde. The quantity of formaldehyde is generally greater in dew than in rain water. The origin of the formaldehyde in

dew seems to be the photo-oxidation of organic matter present on the surface of the soil.

We have carried on numerous experiments by exposing aqueous suspensions of chlorophyll or carotene to sunlight and air in the presence or absence of carbonic acid and we have obtained greater amounts of formaldehyde in the presence of carbonic acid than in its absence. It seems that the energy obtained in the partial photo-oxidation of chlorophyll or carotene is utilized in the formation of formaldehyde from carbonic acid.

A Theory of Carbon Assimilation

The following appear to be the important steps in carbon assimilation.

- 1) Partial activation of carbon dioxide and water at the leaf surface due to their adsorption by chlorophyll and other plant pigments. It seems that chlorophyll and carotinoids present in the leaf act as photosensitisers and as reducing agents in the photoreduction of carbonic acid.
- 2) Further activation of the adsorbed CO_2 and water by absorption of a part of the energy available from respiration and the oxidation of carotene and the formation of activated carbon dioxide and water as products of respiration.
- 3) Absorption of light by chlorophyll and other pigments and the dissociation of activated water molecules on the leaf surface into H and OH and the reduction of activated carbon dioxide molecules to formaldehyde by the atomic hydrogen produced from the sensitised photolysis of water. The amount of energy required to decompose a gram mole of water into H and OH is the same as that necessary for the formation of a gram mole of formaldehyde from carbon dioxide and water.
- 4) The polymerisation of formaldehyde to reducing sugar.
- 5) The formation of hydrogen peroxide from OH and the rapid decomposition of H_2O_2 into water and oxygen on the leaf surface.

The polymerisation of formaldehyde *in vitro* to reducing sugars is an exceedingly slow process even in presence of light. We have shown that it is accelerated by ferric salts. Moreover, it is known that in the presence of alkali, reducing sugars are formed from formaldehyde. Light accelerates this reaction. How the formaldehyde formed on the leaf surface undergoes rapid polymerisation is still unknown. This theory of carbon assimilation appears to have more experimental evidence in its favour than those of Willstätter, Warburg, Wurmser and others. (Compare Dhar and Atma Ram, J. Indian. Chem. Soc., 1933, 10, 287).

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DISCUSSION

Dr. Mackinney: Comments are restricted to the phase of the problem dealing with the formation of formaldehyde *in vitro* from carbonic acid and bicarbonates. With reference to the conditions required by Baly, the work of Emerson, Zscheile, and Bell definitely substantiate the statement (1) that "no procedure has yet been published whereby conditions for obtaining formaldehyde and carbohydrates *in vitro* can be duplicated in other laboratories." As it has been demon-

strated that the formaldehyde obtained by Usher and Priestley using extracted chlorophyll as a catalyst came from the chlorophyll, the use of organic catalysts (such as methyl orange, methylene blue, etc.), is to be deprecated. The challenge will inevitably be made that the traces of formaldehyde reported come from the catalyst, or from minute traces of impurities, and that the conditions required for this decomposition are those necessarily lacking in a so-called control (i.e. illumination, CO₂, or both). One is tempted to extend this line of thought, to enquire into the necessary significance of a positive test for formaldehyde with Schryver's or Schiff's reagent, when compared with a negative result for the "control". To what extent does it genuinely represent a control? As Spoehr (2) has pointed out, the abundance of conflicting evidence has placed the onus of proof on those who obtain positive results.

A definite step forward has been taken in studying limited regions of the spectrum. The discussion by Professor Dhar of the work of Kirkbride and Norrish, gives promise of at least a partial explanation of some of the contradictory results.

Dr. Spoehr: An understanding of the phenomenon of solarisation can be gained only on the basis of quantitative experimentation. It must be remembered that thus far we have relied largely upon the iodine-starch test as an indication of the presence or absence of starch. It will be necessary to replace this with quantitative determinations of starch. In solarisation, is starch actually absent after a period of illumination, or has some substance been formed which interferes with the starch-iodine test? Recently in our laboratory we have found that some leaves after extraction with ethanol until all the pigments were removed, still gave no test for starch with iodine. When the leaf material had been extracted with petroleum ether the presence of starch was easily determined with iodine.

The careful experiments of the late Dr. Holman on solarisation (Univ. of California Publications in Botany, 16, 139-151, 1930), carried out in our laboratory, demonstrated that this phenomenon does not occur without distinct fading of the green color of the leaf and suggest that the destruction of the chlorophyll may be partly responsible for the inability of the leaf to maintain its starch in those areas which have been exposed to intense illumination.

Interesting in this connection is the observation that increased carbon dioxide supply delays solarisation rather than hastening it. This suggests that solarization is not due to the accumulation of the

(1) J. Am. Chem. Soc., 54, 1688 (1932).

(2) Annual Rev. Biochem., II, 466 (1933).

products of photosynthesis, but rather to a direct reduction in the efficiency of the chloroplasts. This conclusion is also supported by the observation made by Holman, that he was never able to secure solarisation in leaves which were filled with starch at the beginning of the experiment. Although it seems to be well established that an accumulation of the products of photosynthesis tends to inhibit the rate of photosynthesis, evidence is still wanting that this inhibition is of such an order that respiration would completely deplete the stored carbohydrates in the form of starch. Apparently there are conditions under which respiration over-balances photosynthesis even during periods of illumination, but this seems to be due to an inhibition of photosynthetic activity, associated with some disturbance in the photosynthetic apparatus, rather than to an unusual stimulation of the respiratory rate.

That there may be a relation between respiration and photosynthesis has been suggested repeatedly, more recently on the basis of experimental evidence by van der Paauw. As determined by Warburg the photosynthetic process is one of relatively high efficiency. There exists as yet no definite evidence that this relationship is of an energetic nature. The mode of approach to this problem, inaugurated by van Niel with the purple bacteria, offers one of the best means. It seems possible that the two processes are dependent upon the same agent or set of conditions which makes possible the splitting of water into H and OH, resulting in active reducing as well as oxidizing agents.

Dr. van der Paauw: The fact that photosynthesis (Matthaei) shows the same relation to temperature as formaldehyde synthesis from bicarbonate solutions, may be purely incidental. Other vital processes show in the main a similar relation to temperature (curves with an optimum type).

The connection between the temperature coefficients for the conversion of formaldehyde to reducing sugars and the temperature coefficient for photosynthesis is, in my opinion, incidental as well. Miss van Amstel worked under conditions with which physical diffusion processes were limiting the photosynthetic process, whereas it is not quite certain that in Bose's experiments no other factor than temperature limited the speed of the total process. In this respect I may refer to my own investigations. I found different temperature coefficients which were by far higher than 1.2 (see *Planta* 22, 396, 1934).

I disagree with Dhar's opinion that the non-applicability of the Arrhenius relation to photosynthesis in plants is due to the influence of respiration. In the experiments of Warburg a correction has been made for respiration. Fur-

ther, I have shown that with algae the temperature coefficients of photosynthesis and of respiration do not differ very much, when care is taken that the factors light and carbon dioxide are not in the minimum. Finally I have found no evidence for the opinion that a temperature higher than 20° is harmful to the chloroplast.

According to Dhar it is needless to assume that the photosynthetic process involves two reactions because of the fact that many photochemical reactions have a temperature coefficient higher than unity. The current opinion on photochemical reactions with a temperature coefficient higher than unity is that they are chain reactions and involve a pure chemical one. The same, therefore, holds for photosynthesis. The explanation of the low coefficient in weak light and in plants poor in chlorophyll, as given by Dhar, is in my opinion not correct. As remarked above, a correction is always made for respiration. So it is impossible to account for this factor.

Is it quite certain that the law of mass action may be simply applied to the process of respiration? In my experiments the rate of photosynthesis (without a correction being made for respiration) was very constant for rather long periods, proving that respirable substances do not accumulate, though the intensity of the photosynthetic process was very high. Products of photosynthesis probably are excluded in an inactive state (starch). Weevers showed that starch formation from sugars proceeds very rapidly. (*Rec. tr. bot. néerl.*, 28, 400, 1931).

Under optimum light and carbon dioxide conditions photosynthesis and respiration never counterbalance. The rate of respiration amounts to a few percent of that of photosynthesis.

In connection with the increase in respiration of *Cladophora* in light, I should like to hear how the other plants behave under the same conditions. In this respect I also point to my own investigation, in which it was shown that respiration probably increases in light.

Dr. Rollefson: The use of heats of reaction to calculate the wavelength of light which will be effective in causing a particular reaction to occur is entirely unwarranted. If an endothermic reaction occurs, the amount of energy supplied by light absorption may be equal to, greater than, or even slightly less than the heat of the reaction, the only absolute requirement being that the effective light must be absorbed by the system. In the paper under discussion it is assumed that since the reaction



is endothermic to the extent of 112,000 calories, any light of wavelength 2550 Å. or less is capable

of causing this reaction to occur. The mechanism given involves the dissociation of water into H and OH followed by the reaction between H and CO₂. The first step in this process requires absorption of light by water and this does not occur appreciably at wavelengths greater than 1850 Å. We are faced therefore with the situation that formaldehyde is formed by light of wavelength less than 1850 Å. rather than 2550 Å., and decomposed by light of wavelength less than 2800 Å. (Kirkbride and Norrish). With an energy distribution such as we have in sunlight, this would mean that formaldehyde would tend to be decomposed very much faster than it formed until a photostationary state involving very little formaldehyde is reached. The introduction of the reversible reaction



is of no help as the rates of the direct and reversible reactions are negligible under ordinary conditions.

The oxygen-ozone "equilibrium" which is mentioned is not a true equilibrium but is a photostationary state with a much higher concentration of ozone than corresponds to the thermodynamic equilibrium.

These remarks do not deny the existence of formaldehyde in rain water but merely point out that the postulated mechanism is improbable.

Dr. Mestre: Would you have any possible mechanism to suggest for its presence in the rain water?

Dr. Rollefson: I think the origin of the formaldehyde is very uncertain. No evidence has been presented by Dhar to show that it has been formed from the carbon dioxide and oxygen in the atmosphere; he is just assuming that.

Dr. Mestre: A point in which I find myself in general agreement with Dhar is in regard to the possibility of the existence of a photosensitized respiration. As I have already stated in my paper on the photosynthetic system of the chromatophore, I think that the postulation of the existence of such a photosensitized respiration, in addition to the normal dark respiration, is by far the easiest way of accounting for some of the data of solarization.

Dr. Emerson: We have, of course, no conclusive evidence that photosensitized respiration doesn't exist, but we know that if it does exist it must be characteristic of organisms having also a photosynthetic mechanism. If *Chlorella* is cultured in such a way that it grows without chlorophyll, respiration is just the same in light as in darkness. It is well-known that yeast cells have the same respiration in light as in dark.

Prof. Dhar: With regard to the points raised

by Mackinney I have to state that the procedure published in my paper for detecting formaldehyde formed in photosynthesis by exposing potassium bicarbonate solutions to sunlight in quartz vessels, has been repeated in the Dacca University (Bengal, India) Chemical Laboratory and the workers there have confirmed our observations. In no case was formaldehyde detected in the control experiments as well as in the catalysts.

I am indebted to Spoehr for drawing my attention to the work of the late Dr. Holman on solarisation. The distinct fading of the green colour of a leaf associated with solarisation does not affect the explanation of the phenomenon offered by us. The carbohydrates photosynthesised by plants actually disappear by respiration, and thus the phenomenon of solarisation is observed. The delay in the appearance of the phenomenon of solarisation observed with increased carbon dioxide supply may be simply explained from the viewpoint that larger amounts of carbohydrates are formed when the carbon dioxide concentration is increased; and naturally for the disappearance of the larger amounts of carbohydrates formed in photosynthesis by respiration, a greater amount of time will be required; hence the delay in the appearance of the phenomenon of solarisation with increased carbon dioxide supply.

Although the efficiency of the photosynthetic process worked out under artificial conditions by Warburg is high, the observations of Pütter, Miller and others show that under field conditions the efficiency of photosynthesis is not high and varies from 2% to 4% only.

The experiments on the photo-oxidation of carbohydrates, salts of fatty acids, and amino acids reported in my paper show that the amount of formaldehyde formed is greater, the greater the amount of energy obtainable from the photo-oxidation. Moreover, the activity of the plant is said to be a measure of its respiration. Hence it appears that we have to take into consideration the amount of energy set free by respiration in understanding the phenomenon of photosynthesis.

In my book on "Chemical Action of Light" (Blackie & Sons, London, 1931) I have discussed numerous cases of temperature coefficients of photochemical reactions and have shown that the actual value of the temperature coefficient of a reaction depends on the temperature interval of the experiment. The value of the temperature coefficient is higher, the lower the temperature interval of the experiment. The higher values of the temperature coefficients of photosynthesis obtained by van der Paauw than those obtained by van Amstel and Bose are due to the fact that the latter investigators worked at temperatures higher than those investigated by van der Paauw.

When a correction is applied for respiration

the apparent photosynthesis becomes less. According to the view put forward in my paper, temperature affects respiration more than photosynthesis, so the correction of the photosynthetic values for respiration would be more pronounced at higher temperatures. Apparently, therefore, the temperature coefficient will be approaching unity especially when the light intensity is feeble.

It is a well-established fact that under certain conditions, respiration counterbalances photosynthesis even in the presence of light. I have tried to explain this. In order that light may influence respiration, it must be absorbed by the system undergoing respiration. The observation of Emerson that the respiration of *Chlorella* when grown without chlorophyll and of yeast is not influenced by light, may be explained from the

viewpoint that neither of these systems absorbs the incident light appreciably.

I should like to point out to Rollefson that the majority of physicists and physical chemists are still using the heat of a reaction in calculating the effective wavelength for a particular chemical change. If the oxygen-ozone equilibrium is taken to be a photostationary state, the reversible reaction $\text{H}_2 + \text{CO} \rightleftharpoons \text{H}_2\text{CO}$ can also be considered as a photostationary state.

I am very pleased to find that Mestre is in general agreement with my view that respiration may be a photosensitised reaction. It gives me great pleasure to find that the mechanism of photosynthesis advocated by me, that the photosensitised decomposition of water H_2O (activated) $\rightarrow \text{H} + \text{OH}$ is the first stage, has been generally accepted.

THE KINETIC MECHANISM OF PHOTOSYNTHESIS

DEAN BURK AND HANS LINEWEAVER

INTRODUCTION

Historical Perspective. It is proposed to present in this paper a general kinetic mechanism of photosynthesis which will embody a large number of new and important experimental facts established within the last five years, in addition to findings long demonstrated. The physico-chemical mechanism offered will provide comprehensive, but conservative, unification in the field of photosynthesis. The majority of mechanisms proposed heretofore in connection with various aspects of photosynthesis have almost invariably contained, as essential features, unsupported, *ad hoc* hypotheses which have definitely limited their probability, and hence their general applicability and value. Several mechanisms have restricted themselves to certain well-established facts, but have neglected, or have been at variance with, other available critical data. This has commonly been the case where the supporting investigations have been concerned chiefly with one particular aspect of photosynthesis, such as the organic chemistry of the plant pigments, or fluorescence phenomena, or induction, or energy relations, or, in general, too few of the known major variables.

Very few mechanisms have been concerned with detailed kinetic expressions based upon clearly defined physico-chemical schemata. Attention previous to 1905 was centered mainly upon the three "cardinal conditions" of minimum, optimum, and maximum, and, for some fifteen years following, upon the essentially qualitative, Blackman Principle of Limiting Factors (8), or upon the modification of this principle with respect to differentiation between relative and absolute minima. It is chiefly since about 1920, commencing with the outstanding, and to date unsurpassed, work of Warburg, that quantitative data covering many variables have been treated in terms of catalytic reaction kinetics. Most of the conceptions developed prior to this period have already served their period of usefulness, and have passed into discard in this sense. It is only reasonable to suppose that the outlooks presented in this symposium will eventually, in the course of a decade or so, have shared the same fate. It would, of course, be unhealthy to assume a very different attitude; in the problem at hand, the emphasis definitely lies on usefulness, or means to ends, not on ultimate correctness. In the words of G. N. Lewis (30, p. 6),

"The scientist is a practical man and his are practical aims. He does not seek the *ultimate* but the *proximate*. He does not speak of the last

analysis but rather of the next approximation. His are not those beautiful structures so delicately designed that a single flaw may cause the collapse of the whole. The scientist builds slowly and with a gross but solid kind of masonry. If dissatisfied with any of his work, even if it be near the very foundations, he can replace that part without damage to the remainder.

"The theory that there is an ultimate truth, although very generally held by mankind, does not seem useful to science except in the sense of a horizon toward which we may proceed, rather than a point which may be reached."

Scope of Proposed Mechanism. The mechanism advanced will define the nature and *minimum* number of light and dark reactions required to represent accepted knowledge and herein presented deductions concerning the action of the best known of the external and internal factors involved in photosynthesis by commonly studied chlorophyllous plants. Photosynthesis will be represented by a reasonably unique, physico-chemical mechanism involving at least the five factors, carbon dioxide, light, temperature, chlorophyll, and an enzymic Blackman component, the last two being internal. Whereas the external factors are generally limiting only in intensity, the internal factors might exhibit both an intensity and a capacity limitation. The capacity limitation on the part of chlorophyll has long been demonstrated, in any case with certainty since the work of Emerson (14, 15), but the Blackman component has hitherto been treated as limiting, experimentally, only in intensity (concentration). It will be a special feature of this paper to indicate the capacity limitation (saturation by chlorophyll intermediate) on the part of the Blackman component. The view that photosynthesis can be represented by a light and a dark reaction will be discarded in favor of a minimum of three or four light reactions, which may be grouped kinetically into one, and three recognisable dark reactions, of which the light reactions can be neither the first nor the last.

Each detail of the "minimum" kinetic mechanism developed will be supported by one or more lines of evidence, and will be inconsistent in no particular with information and understanding at hand. The mechanism is to be regarded as a fairly rigid skeleton mechanism, but one easily capable of undergoing flexible, consistent expansion as further experimentation may indicate. As a tool for the simplification and unification of previous results, and by virtue of its concise kinetic, mathe-

mathematical expression of fact, it should be useful in (1) suggesting experimentation, particularly in connection with new variables not already treated; (2) testing mechanisms developed from other points of view, chiefly non-kinetic; and (3) orienting interpretation of independent, often minor, findings not sufficiently interrelated for the present to be critical with respect to any mechanism. No attempt will be made, in the space available, to enter into any detail concerning the last two considerations, but a number of critical experiments suggested by the kinetic equations developed will be outlined to provide important extension of present information, and, in instances, testing of the mechanism on the basis of its predictions.

The minimum mechanism proposed will be seen to resemble in various details certain mechanisms advanced by previous investigators, and indeed, to derive much of its support from them, especially in the cases of the mechanisms of Willstätter and Stoll (45), Warburg and Uyesugi (43), Shibata and Yakushiji (36), Ghosh (22), James (25), Emerson and Arnold (16), van den Honert (37), and Muller (34). In this connection, however, it is desired to stress the point that the supporting, deductive evidence in the present case is in several important respects original and uniquely determinate. Thus the CO_2 -chlorophyll complex suggested by Willstätter and Stoll (45) on the basis of direct chemical evidence may be totally unrelated to the CO_2 -chlorophyll complex indicated by kinetic data. The resemblances appearing may thus often be superficial. A similarity in final form is therefore not so significant as a comparison of the actual deductions involved. The mechanism offered is definitely at variance, in one or more important aspects of omission or commission with the following mechanisms which we have examined in detail: Baly and Morgan (7), Emerson and Green (18), Baly (5, 6), Kautsky (26), Conant, Dietz, and Kamerling (13), Adams (1).

The proposed mechanism presents new features as just indicated, and, in particular, derived and tested reaction kinetics. The kinetics developed will express the rate of photosynthesis as a function of steady states and equilibria affected by the commonly recognised external and internal factors. Limitation of space available here requires postponement of consideration of the rate in continuous light as a function of time (induction), the rate in flashing light as a function of insufficient dark periods and low light intensities and carbon dioxide pressures, fluorescence, wavelength and energy relations, carbohydrate production, and organic chemistry of the plant pigments.

Finally, because of the comparative singularity of the process of photochemical reduction in Nature, in being limited essentially to plants, it is believed preferable, once a fundamental mechanism of photosynthesis has been established with some generality, to regard it as holding in all (chlorophyllous) plants until demonstrated otherwise. Experience shows that, with a process so highly specific, it is unlikely that Nature would develop a wide variety of fundamentally different paths. One may well expect a "Unity in Nature", as shown in various ways so ably by Kluyver and members of his school. It is better not to take the view, *a priori*, that each species or group of plants is a new problem in important qualitative particulars. In this connection it is desirable, if possible, to have available as comprehensive a kinetic treatment as factually permissible, which will, under differing limiting conditions, in different plants, reduce by loss of terms to differing simpler kinetic equations, all consistent with the same fundamental mechanism.

Goodness of Fit. It became very clear in the course of our analysis, that it is easy to over-emphasize the importance of an excellent or surpassing agreement of data with mechanism, when a small number of variables, two or at most three, are under consideration. It is usually necessary to assume limiting conditions often not actually obtaining, and such fits frequently represent, in spite of all desire to the contrary, a considerable degree of fortuitousness. In the complex systems under consideration, seemingly excellent fits will often produce deceptive aspects of simplicity, tending to obscure approximations in a manner that is perhaps gratifying, but assuredly immature. It is well known that an equation in one or two independent variables, in which there are several arbitrary constants, can express such a variety of experimental data that there is often no practical significance to such a fit. These statements are not to be regarded as any attempt to lessen the value of accurately determined constants, but to indicate that, in a large problem like photosynthesis, their interpretation requires much of the approximative, statistical outlook, and a judicious balancing of probabilities. As Clerk Maxwell has said (11, p. 178), "Experiment furnishes us with the values of our arbitrary constants, but only suggests the form of the functions." We know now, of course, that the distinction implied is more relative than absolute (32, p. 229). In any biological system there are usually too many variables operating to allow of "absolute proof" of the nature of the processes underlying observed quantitative relations, and hence decisions must often be based on general probabilities. Nevertheless, as A. J. Clark (12)

points out, "However complex an overall process may be, whether a biological or purely chemical reaction, what one measures usually, is far more often than not, but one or at most two or three elements of the complexity; the problem then, is not to expect a complete description of the complete overall process, but to find out what element or elements it is that are being measured; and then possibly further, having found these to change conditions so that not these same elements, but new elements are measurable; and so on till all elements of interest have been investigated so far as available technique will permit. As a matter of fact, certain particular elements of an overall process will probably in general be of more interest than the process as a whole." The problem of photosynthesis probably represents an exception to the last statement; at any rate, such a view will be adopted in this paper.

As more factors become studied and introduced into a broad problem, qualitative tests, involving analysis of general shapes and tendencies of curves, assume more significance, and exact quantitative fits still more, even. In this sense, a complication of analysis should be welcomed. It is then desirable to proceed in the following manner when practical. (1) Evaluate certain constants independently of the kinetic treatment (such as by determining the dissociation constant of the CO_2 -chlorophyll complex by direct gasometric measurement in the dark—without involving photosynthesis. (2) Obtain families of curves where the velocity of photosynthesis is studied as a function of two or more variables in a parametric manner. The expression of the data for each new parameter studied may differ only in those constants involving the parameter, and furthermore they may differ only in a definite manner so that the constants are no longer arbitrary. (3) Obtain certain constants by employing practically limiting conditions. In this manner one should be able to reduce the number of arbitrary constants to zero, one or two, and if a fit is still obtained over the whole range of the several factors, the proposed mechanism may be expected to be highly significant. In these connections it is perhaps well to recall the words of Ingenhousz, the discoverer of the role of light in carbon dioxide reduction by plants, "Natural knowledge can make but very slow progress in the hands of those who have not patience and assiduity enough in pursuing one and the same object, till they discover some things undiscovered before; or till they find that the difficulty of the undertaking surpasses their abilities."

It is unfortunately true that, essentially without exception, there are no photosynthetic data which have been obtained and replicated in a

manner that would permit adequate determination of the statistical probability of the functions obtained, by some such method as Pearson's Chi Test, involving knowledge of the standard deviations and weightings as functions of the variables (32, p. 229; 10, p. 38; and cf. James, 25, p. 32). This same consideration has recently been urged in regard to many physical data, in several papers by W. E. Deming, and is only the more pressing in regard to available photosynthetic data of a kinetic nature.

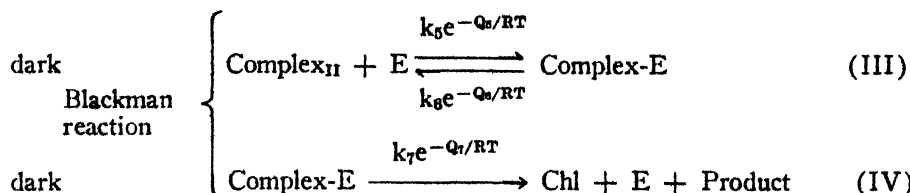
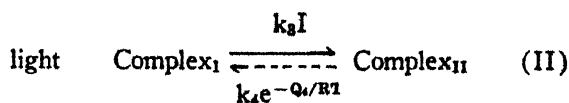
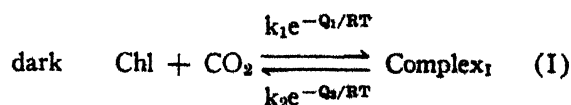
For the present, then, a fairly good qualitative agreement between data and rigorously defined mechanism involving many variables would appear to deserve more attention, in general, than an excellent fit obtained with one or two variables on particular data which will not readily submit to close examination for either random or systematic errors. We are only now on the verge of an era wherein the greatly more desirable, accurate quantitative fits with many variables should be obtainable. In the words of G. E. Briggs (9, p. 39), "The data for photosynthesis are so numerous that the time seems ripe for the more precise formulation of theories, many of which have been but vaguely suggested and rarely put to a quantitative test. The process of formulation, testing, and rejection or refinement, must necessarily be tedious —." It is well, too, to recall Priestley's remark, "*Theory and Experiment* necessarily go hand in hand, every process being intended to ascertain some particular hypothesis, which in fact, is only a conjecture concerning the circumstances or the causes of some natural operation" (35, p. 259); or, in the later words of Rashevsky, "— in its future development the theoretical research (in biology) will have to go hand in hand with the experimental, and ask of the latter information which may not yet be available, and for which the experimental scientist would even not have looked." (23, p. 6).

The rather extended nature of the introductory discussion provided in this paper has arisen in part from the fact that in the course of our studies we have come to have the opinion forced upon us that the broad problem of photosynthesis involves a great many matters of scientific advocacy going beyond purely experimental aspects.

Nomenclature and Conventions. The following significance of symbols will be assigned throughout this paper: (CO_2) will represent the measured concentration of carbon dioxide, without necessary commitment as to hydration or ionic dissociation; (Chl_t) the concentration of total "effective" chlorophyll, reckoned as amount per unit volume of cells, without regard to either molecular weight (molecules per photosynthetic unit (4)) or possible non-homogeneity of distri-

bution; (Chl), concentration of free (uncombined) "effective" chlorophyll; I, the incident light intensity, normally assumed to be essentially equal to the transmitted light intensity; T, the temperature; (E) the concentration (amount per unit volume of cells) of a free enzyme concerned in the Blackman reaction; and y the experimentally observed steady state rate of photosynthesis. Physical diffusion, frequently involved, or other incidental factors (chemical reactions) affecting the availability of CO_2 as well as variable light

section with the following variables: T, I, (CO_2), (Chl_t), (E), specific and indifferent narcotics, and intermittent illumination.



intensity (Beer's law) are here regarded as comparatively uninteresting phenomena incidental to the main chemical mechanism under consideration. *These factors should be removed experimentally wherever possible or at least the data obtained should be validated by the correct analytical treatment;* such phenomena where concerned will be specifically mentioned separately. H_2O will not be considered as a variable since no critical data as to its kinetic role are available. The importance of such physiological factors as chromatophore aggregation and orientation, stomatal opening, pH, variation of experimental material, species etc. are not to be neglected, of course, when definitely demonstrated. The defined product of photosynthesis will be O_2 gas without necessary commitment as to any carbohydrate-like material. The point of elimination of O_2 though placed in step (IV) is not definite. Except where otherwise indicated, the rate of photosynthesis in flashing light will refer to experiments in which the intervening dark periods were sufficiently long to give maximum yield. The kinetic treatment will be based in part upon methods outlined earlier (10a) and the data will be taken chiefly from studies on lower green plants, with a certain emphasis on *Chlorella (pyrenoidosa)*, *Hormidium*, *Fontinalis* and *Gigartina Harveyana*.

PROPOSED MECHANISM

Schema A. This schema represents a reasonably general minimum mechanism which is consistent with chemical and kinetic experience and is deduced from the several major facts now established for many chlorophyllous plants in con-

Kinetics. Let $k'_1 = k_1e^{-Q_1/RT}$, $k'_2 = k_2e^{-Q_2/RT}$, etc., with k_3I temperature insensitive. The total concentration of chlorophyll, free and combined, is

$$(\text{Chl}_t) = (\text{Chl}) + (\text{Complex}_I) + (\text{Complex}_{II}) + (\text{Complex-E}) \quad 1$$

and that of the enzyme is

$$(\text{E}_t) = (\text{E}) + (\text{Complex-E}). \quad 2$$

The steady state rate of photosynthesis is

$$y = k'_1(\text{Chl})(\text{CO}_2) - k'_2(\text{Complex}_I) \quad \text{from (I)} \quad 3$$

$$= Ik_3(\text{Complex}_I) \quad \text{from (II)} \quad (k'_4 \text{ negligible}) \quad 4$$

$$= k'_5(\text{Complex}_{II})(\text{E}) - k'_6(\text{Complex-E}) \quad \text{from (III)} \quad 5$$

$$= k'_7(\text{Complex-E}) \quad \text{from (IV)} \quad 6$$

From Eq. 6

$$(\text{Complex-E}) = y/k'_7 \quad 7$$

From Eqs. 2, 5, and 7

$$(\text{Complex}_{II}) = y(k'_7 + k'_6)/k'_5[k'_7(\text{E}_t) - y] \quad 8$$

From Eq. 4

$$(\text{Complex}_I) = y/k_3I \quad 9$$

From Eqs. 3 and 9

$$(\text{Chl}) = y(k_3I + k'_2)/k'_1k_3(\text{CO}_2)I. \quad 10$$

Substituting Eqs. 7, 8, 9, and 10 in Eq. 1, one obtains

$$y = \frac{k'_1k_3(\text{Chl}_t)(\text{CO}_2)I}{k'_1k_3(\text{CO}_2)I[1/k'_7 + (k'_7 + k'_6)/k'_5\{k'_7(\text{E}_t) - y\}] + k'_1(\text{CO}_2) + k_3I + k'_2} \quad 11$$

$$\text{Let } D = 1/k'_7 + (k'_7 + k'_8)/k'_8\{k'_7(E_t) - y\} \quad 12$$

When $(E) \doteq (E_t)$ or $y \ll k'_7(E_t)$, corresponding to the Blackman factor being unlimiting in capacity (negligibly saturated), then

$$D = [k'_8(E_t) + k'_8 + k'_7]/k'_7k'_8(E_t), \quad 13$$

a constant.

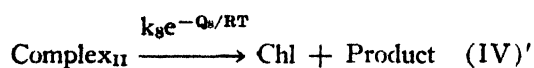
Substituting D in Eq. 11, letting $k'_1 = k_1e^{-Q_1/RT}$ etc., and dividing numerator and denominator by $e^{-Q_1/RT}$, one obtains

$$k_1k_8I(\text{CO}_2)(\text{Chl}_t)$$

$$Dk_3k_1I(\text{CO}_2) + k_1(\text{CO}_2) + k_8Ie^{Q_1/RT} + k_2e^{(Q_1-Q_2)/RT}$$

14

Schema B. This schema is identical with A in reactions (I) and (II) but substitutes for (III) and (IV) the reaction

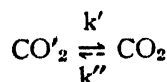


so that

$$y = \frac{k_1k_8I(\text{CO}_2)(\text{Chl}_t)}{k_3k_1I(\text{CO}_2)k_8e^{-Q_8/RT} + k_1(\text{CO}_2) + k_8Ie^{Q_1/RT} + k_2e^{(Q_1-Q_2)/RT}}, \quad 15$$

thus D in Eq. 14 is replaced by $1/k_8e^{-Q_8/RT}$. This mechanism is consistent with all data that do not involve saturation of the Blackman enzyme, if k_8 is allowed to vary somewhat from experiment to experiment.

Data derived under the condition of mechanical diffusion or its equivalent may be both recognized and made intelligible in view of the following considerations. Let CO'_2 be the external and CO_2 the internal carbon dioxide. We may then write as before



and

$$y = k'(\text{CO}'_2) - k''(\text{CO}_2) \quad 16$$

$$\text{when } (\text{CO}_2) = [(\text{CO}'_2) - y/k']k'/k''. \quad 17$$

so that Eq. 14 may be altered in a definite manner to include diffusion in the treatment. When making the linear test-plots with respect to (CO_2) (suggested later) when diffusion is limiting the quantity $[(\text{CO}'_2) - y/k']$ may be used conveniently in place of (CO_2) (i.e. $[(\text{CO}'_2) - y/k']/y$ is plotted against $[(\text{CO}'_2) - y/k']$, see Table I and Eq. 25).

The (CO_2) may be corrected for respiration in schema A by an equation analogous to that of James (25, p. 23) if with James we let r represent a constant rate of supply of CO_2 by respiration so that Eqs. 16 and 17 become

$$y = k'(\text{CO}'_2) - k''(\text{CO}_2) + r \quad 18$$

and

$$(\text{CO}_2) = k'(\text{CO}'_2)/k'' - (y - r)/k''. \quad 19$$

James, treating the steps, including diffusion, that occur before the Blackman reaction, postulated essentially the same mechanism as schema A (cf.

also Maskell, 33). He does not however give a complete general kinetic equation, either explicit or implicit. With Ghosh (22) (see later) he gives treatments depending on a Blackman factor practically unlimited in capacity. Also he speaks of the light-formed intermediate (by consecutive light absorption) corresponding to Complex_{II} , as

a peroxide. Table I, figure 3, and figure 5 of his paper (25) demonstrate the agreement of data with this mechanism, particularly with respect to diffusion. In his section on temperature James apparently was unaware that the general kinetic equation (not reported by him) may be used to show what conditions are necessary to cause different reactions to be controlling (cf. p. 36 of his paper and our Table II).

The following equations are explicit solutions of equation 14 for (CO_2) in the absence and presence of diffusion limitation respectively, when $k' = k''$ and $r = 0$.

$$(\text{CO}_2) = \frac{yk_m - y^2}{y^2k_n - yk_p + k_q} \quad \text{no diffusion} \quad 20$$

$$(\text{CO}'_2) = \frac{y}{k'} + \frac{yk_m - y^2}{y^2k_n - yk_p + k_q} \quad \text{with diffusion} \quad 21$$

Where $k_m = k'_7(E_t)$

$$k_n = k'_1(k'_7 + k_8I)/k'_7(k'_2 + k_8I)$$

$$k_p = \frac{k'_1 k_8 I [k'_5 (E_t) + k'_6 + k'_7 + k'_5 (\text{Chl}_t)] + k'_1 (E_t) k'_5 k'_7}{k'_5 (k_8 I + k'_2)}$$

$$k_q = \frac{(\text{Chl}_t) k'_7 (E_t) k_8 I k'_1}{k_8 I + k'_2}$$

In the presence of diffusion but when $y \ll k'_7 (E_t)$

$$(\text{CO}'_2) = \frac{y k_r - y^2/k'}{k_t - y} \quad 22$$

where

$$k_r = \frac{[k_8 I + k'_2 + k'_1 k_8 I (\text{Chl}_t)/k'] k'_7 k'_5 (E_t)}{[k'_5 (E_t) + k'_6 + k'_7] k_8 k'_1 I + k'_1 k'_7 k'_5 (E_t)}$$

$$k_t = \frac{k_8 I (\text{Chl}_t) k'_7 k'_5 (E_t)}{[k'_5 (E_t) + k'_6 + k'_7] k_8 I + k'_7 k'_5 (E_t)}$$

from which it may be seen that at high values of I and at low values of y (i.e. low values of (CO'_2))

$$y = (\text{CO}'_2) k' k'_1 (\text{Chl}_t) / [k'_1 (\text{Chl}_t) + k'] \quad 23$$

and if $k'_1 (\text{Chl}_t) \gg k'$ then

$$y = k (\text{CO}'_2), \quad 24$$

which shows the significance of the first linear portion of y vs. (CO'_2) plots where diffusion is limiting.

$$(\text{CO}_2)/y = [1/k_8 I n + D/n] (\text{CO}_2) + 1/k'_1 n + k'_2/k_8 I k'_1 n \quad 25$$

$$I/y = [1/k'_1 n (\text{CO}_2) + D/n] I + 1/k_8 n + k'_2/k_8 k'_1 n (\text{CO}_2) \quad 26$$

Evaluation of Constants (Equation 14). Employing the value of D given by the assumption in Eq. 13 or employing schema B, Eq. 14 has essentially only the variables (CO_2) , I , T and (Chl_t) . If T , (Chl_t) and (E_t) are maintained constant and the external variables (CO_2) and I varied to obtain a family of parametric curves, one may evaluate each of the constants in Eq. 14 in terms of (Chl_t) by direct analytical treatment of the experimental data.

The data of Harder (22a) have been treated in the proposed manner by Ghosh (22). Although some doubt has been cast upon the validity of these data it should be pointed out that since both I and (CO_2) have been varied over a significant range they represent the most comprehensive data available for this treatment. The paper of Ghosh, which seems to have been largely overlooked, undoubtedly takes a leading rank in the matter of previous kinetic treatment of photosynthetic data.

Table I shows the types of linear plots, and the significance of the slopes and intercepts that may be employed in evaluation of the constants of Eq.

TABLE I. *Evaluation of Constants for Schema A (D Constant) by Linear Plots*

Column No.	1	2	3	4	5	6
Type of plot	$(\text{CO}_2)/y$ vs. (CO_2)	I/y vs. I	Slope (1) vs. $1/I$	Slope (2) vs. $1/(\text{CO}_2)$	Intercept (1) vs. $1/I$	Intercept (2) vs. $1/(\text{CO}_2)$
Slope	$1/k_8 I n^* + D/n$	$1/k'_1 (\text{CO}_2) n + D/n$	D/n	D/n	$1/k'_1 n$	$1/k_8 n$
Intercept	$1/k'_1 n + k'_2/k_8 k'_1 I n$	$1/k_8 n + k'_2/k_8 k'_1 (\text{CO}_2) n$	$1/k_8 n$	$1/k'_1 n$	$k'_2/k_8 k'_1 n$	$k'_2/k_8 k'_1 n$
Variable(s) = 0 at intercept	(CO_2) only	I only	Both (CO_2) and I	Both (CO_2) and I	Both (CO_2) and I	Both (CO_2) and I

* $n = (\text{Chl}_t)$

Table I gives an analytic method of evaluating the constants in Eq. 14 by linear plots when diffusion is not limiting and when D is a constant (Blackman factor of unlimiting capacity). The forms of the "single reciprocal" plots corresponding to columns 1 and 2 in Table I are:

14. In the case of each of the six plots the linearity and the existence of a positive intercept, when the independent variable equals zero, constitute tests of the mechanism. It is evident from the table that two independent evaluations of D/n (where $1/D = k'_4$ in graph and $n =$

(Chl_t), k_8n and k'_1n and two evaluations of k'_2n depending on the values of k_8n and k'_2n , may be made so that additional tests of the mechanism are given by this required consistency. The constants so determined may be employed to make comparisons between y_{calc} and y_{obs} .

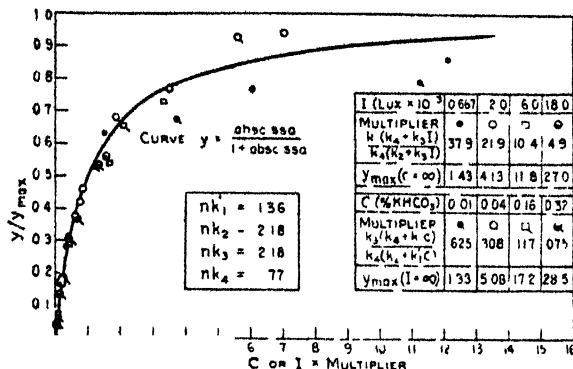


Figure 1. Superimposition of Harder's data (22a). The solid line is the theoretical curve consistent with the constants and the points are the transposed observations. The abscissa is either $C \times \text{Multiplier}$ or $I \times \text{Multiplier}$ as indicated in the inset.

Fig. 1 shows the data of Harder united in a single plot. This was done by noting that when I is constant, Eq. 14 takes the form

$$y = y_{\text{max}} a(\text{CO}_2) / [1 + a(\text{CO}_2)] \quad 27$$

and when (CO_2) is constant, the form

$$y = y'_{\text{max}} a'I / (1 + a'I) \quad 28$$

$y_{\text{max}} = k_8n / (1 + Dk_8I)$, $y'_{\text{max}} = k'_1n(\text{CO}_2) / [1 + k'_1D(\text{CO}_2)]$ and a and a' are the "multipliers" of Fig. 1. If one plots y/y_{max} against the appropriate multiplier x (CO_2) or multiplier $x I$ the curves should superimpose (cf. Fig. 1). Figure 1 also gives the values of the constants and y_{max} reported by Ghosh and the values of the multipliers calculated therefrom and used by us in superimposing the data.

The degree of saturation with respect to either CO_2 or light when the other variable is considered infinite, may be calculated consistent with this treatment in the following manner: when both (CO_2) and I are infinite $y_{\text{max}} = y'_{\text{max}} = n/D = 77$. The highest value of y_{max} obtained was $(2.18)18 / [1 + (2.18)18/77] = 26$, which corresponds to only 34% saturation with respect to light at infinite CO_2 concentration. Similarly, the highest experimental value of y'_{max} obtained was 28, which corresponds to only 36% saturation with respect to CO_2 at infinite light intensity. The 34% saturation with respect to light means that when (CO_2) is infinite 34% of the Chl_t

would be in the form $\text{Complex}_{II} + \text{Complex-E}$ and 66% in the form Complex_I , the ratio $\text{Complex}_{II}/\text{Complex-E}$ is essentially constant (a result of the assumption regarding the Blackman factor Eq. 13). The 36% saturation with respect to CO_2 means that when I is infinite 36% of the Chl_t is in the form $\text{Complex}_{II} + \text{Complex-E}$ and 64% in the form Complex_I .

It is desirable to point out five facts with regard to Harder's data and the fit obtained in Fig. 1: (1) The linearity obtained in the various plots of Table I was not convincing, due in part to lack of data (only four points per plot), and so it does not seem pertinent to report these plots. (2) The combined data do not show any pronounced trend but the high probable error does not allow us to be too critical. In triplicate experiments reported by Harder the percent deviation varied from 1 to 8 with an average of about 3.5%. Of the 32 calculated points only 5 deviated from the observed by more than 8% and the average deviation was only about 4%. (3) The superimposition of the various curves has been carried out in a consistent manner in contrast to an *ad hoc* choice of multipliers which would superimpose any given related or unrelated rectangular hyperbolae. (4) In making such an analytical analysis it is evident that experimental data that attain considerably greater degrees of saturation with respect to both (CO_2) and I than 35% would be highly desirable. (5) The fit of a family of curves rather than individual curves obviously possesses much greater significance with regard to a proposed mechanism. Owing to the uncertainty of the data the results of this treatment neither disprove nor lend paramount support to the proposed mechanism. The possible use of this analytical method, however, points out the desirability of possessing adequate quantitative data.

AGREEMENT OF MECHANISM WITH DATA

We shall consider in this section the facts from which the individual nature as well as the interrelation of the reactions in schema A were deduced.

Reaction (1). The existence of a temperature coefficient at low values of (CO_2) where $y \propto (\text{CO}_2)$ is evidence for compound formation by CO_2 of some type. The existence of a chemical reaction before the light reaction with respect to a given CO_2 molecule is further confirmed by the observation that indifferent narcotics inhibit at both high values of I (where the Blackman reaction is limiting) and low values of I (where the light reaction is limiting (38, 39)). The observation that indifferent narcotics (3) and ultraviolet light (2) decrease the maximum yield per flash, when adequate energy is contained in a flash, is

evidence that they affect the complex formed before the light reaction (i.e. Complex_I) (see also 16, the change in the dark time with thymol may be due to the Blackman reaction also being affected, cf. 29). Evidence against CO₂ entering the Blackman reaction by collision, hence for the formation of a CO₂ complex before the light reaction, is given by the fact that the dark time required for half maximum yield in flashing light experiments is essentially independent of (CO₂) (16, p. 411). A similar argument holds for chlorophyll (16, p. 413).

The hyperbolic nature of the y vs. (CO₂) curves (linearity of the (CO₂)/ y vs. (CO₂) and $1/y$ vs. $1/(CO_2)$ plots, data of Warburg (39), van der Pauw, (38), Emerson and Arnold (16), Emerson and Green (19), James (25)) indicates, on the basis of experience, that CO₂ combines unit for unit with a constituent in the plant that is limiting in amount. By analogy with the kinetics of enzymes such as invertase and amylase (extracellular) (31) and nitrogenase (intracellular) (32) this would be interpreted as probably being due to reversible combination. Two additional lines of evidence are available to support the proposed reversibility in step (I): (1) It is seen in Table I that the value of the intercept in the (CO₂)/ y vs. (CO₂) plot should vary with I if k'_2 has a significant value but should be independent of I if $k'_2 = 0$. The small amount of data available indicates that k'_2 is not zero (31), (39), (38), (16), (22), (19), (24). (2) Emerson and Arnold (16), in flashing light experiments where sufficient dark times were allowed for completion of the Blackman reaction and for equilibration of CO₂ with the plant constituent if an equilibrium occurs, or for complete reaction if reaction (I) were irreversible, obtained yields per flash which are approximately a hyperbolic function of (CO₂) (cf. footnote c, Table III). Although the maximum yields as a function of (CO₂) do not give an excellent fit to a hyperbola (the experimental error is relatively high), the value of k'_2/k'_1 obtained is at least comparable with those obtained in continuous light with I small or zero (extrapolated) (see Tables III and IV). Direct evidence with regard to the amount of "effective" chlorophyll and confirmation of the existence of such an equilibrated Chl-CO₂ complex *in vivo* might be obtained if CO₂ uptake by e.g. *Chlorella* were measured in the dark. Four criteria of significance exist for such data: (1) the range of pressures employed must correspond to that sensitive in photosynthesis (the dissociation constants evaluated must be comparable in the two cases) and (2) the uptake must parallel the "effective" chlorophyll as determined by flashing light experiments (17, Fig. 2), (3) the uptake

should be reversible and (4) the uptake should be sensitive to indifferent narcotics. The variation of Chl_{effective} may be obtained by method of culture, ultraviolet light inactivation or other means.

Reaction (II). This reaction is considered irreversible. The evidence is obtained from flashing light experiments of Emerson and Arnold (16). If after a light flash a significant part of Complex_{II} were able to return to Complex_I directly, instead of proceeding through the Blackman reaction, the yield per flash (with sufficient dark time) would vary with variation in the rate of the Blackman reaction unless the coincidence occurred that the reverse of (II) varied in exactly the same manner. The irreversibility is thus supported by the observation that the maximum yield per flash remains constant when the rate of the Blackman reaction is varied by temperature and HCN (16, Figs. 8 and 9).

The fact that y vs. I curves are not concave upward requires that the light enter in a first order manner (not according to I^2 or I^3 etc.). Additional evidence is given by the observation that the maximum yield per flash is independent (within 9%) of I so long as the energy per flash remains constant (17, p. 202). This conclusion may be reconciled with the quantum efficiency of four obtained by Warburg and the minimum thermochemical (first law) quantum efficiency of three by: (1) postulating four consecutive light reactions, possibly interposed by sufficiently rapid dark reactions, with one reaction being considerably the slower or (2) postulating four consecutive or independent (not simultaneous) light absorption processes (cf. James 25, p. 20). Demonstrated separability or inseparability of the light reactions (e.g. by very short light flashes) would be highly pertinent. The first order nature of reaction (II) with respect to a chemical constituent (i.e. Complex_I) has been indicated by a small amount of data obtained by Emerson and Arnold (17, Figs. 1 and 4, linearity of $\ln(K - M)/K$ vs. energy plot where K = maximum yield, and M = yield per flash).

The kinetic treatment (Eq. 14) assumes that the light intensity is uniform throughout the plant and would need alteration were this not the case (e.g. were the experiment performed in a manner that allowed the light intensity to decrease logarithmically in accordance with Beer's law, see James (25), Brackett (this volume), and others). The following rather unsatisfactory exponential equation implicit in y results from schema A if it is assumed that the light intensity varies according to Beer's law and that neither the Blackman component nor diffusion is limiting

$$y = k_3 I \left[1 - \exp. \left(-k k'_1 (\text{CO}_2) \left\{ (\text{Chl}_t) - y (k'_7 + k'_6) / k'_5 [k'_7 (E_t) - y] + 1/k'_7 + 1/k'_1 (\text{CO}_2) \right\} / \{k'_1 (\text{CO}_2) + k'_2\} \right) \right] \quad 29$$

where the first y term in the exponent will drop out at high values of (CO_2) if $k'_7(E_t) \gg y$. The function of y against (CO_2) is of a mixed hyperbolic-logarithmic type, demonstrating the desirability of experimentally eliminating this factor.

Reactions (III) and (IV). Evidence that chlorophyll is involved in the Blackman reaction is based on the fact that whereas decrease in y by lowering the value of I removes the characteristics of the Blackman reaction (39) (i.e. temperature and HCN sensitivity), decrease in y by lowering the chlorophyll concentration does not (15). The temperature coefficient at high and low chlorophyll concentrations is not greatly different (15). In schema A reactions (III) and (IV), which are based partly on the general belief that the Blackman reaction involves an enzyme (41, 45), are employed to account for the simultaneous observations of a concave downward increase in y with (Chl_t) in continuous light (17, Fig. 3)* and the linear increase in yield per flash with (Chl_t) in flashing light (17, Fig. 2). In view of the complex nature of the system one test is, of course, little more than a bare indication and it becomes desirable that at least the following tests concur in their implications. (1) The above. (2) Since indifferent narcotics have been shown to decrease the amount of effective chlorophyll (15, p. 414-5, cf. also 38, 39), if saturation of an enzyme causes the concavity in test (1) use of indifferent narcotics should remove the concavity, while if decrease in (E_t) causes the concavity the curvature should be unaffected by the narcotics. (3) The apparent order of the Blackman reaction, as indicated by flashing light experiments at high values of I and (CO_2) , should decrease from first order** as (Chl_t) is increased. Tests (2) and (3) neither demand that (E_t) remain constant nor that

the same fraction of Chl_t be effective in photosynthesis (it requires only that the "effective" concentration of chlorophyll increase and of course be very large compared with (E_t) , which would seem reasonable). (4) Arnold (2) by using the effect of ultraviolet light on photosynthesis and the conclusion that the light reaction is first order (see *Reaction II*) shows that one can obtain evidence of the order of the Blackman reaction by comparing the survival ratios in continuous and flashing light. The order with respect to Complex_{II} as indicated by this method should vary from first to zero according as (Chl_t) increases. (5) When E is about saturated y should not be a hyperbolic function of I and (CO_2) (i.e. the $y/(\text{CO}_2)$ vs. (CO_2) plots should be concave upward). (6) When I and (CO_2) are large $\log y$ should be a curved function of $1/T$ over the range that is physiologically unharmed, the degree of curvature depending on the magnitude of ΔH etc. (see Table II).

Of the above proposed tests for E saturation data are available for (1), (4) and (6). Considering (6), most of the temperature data at high values of I and (CO_2) give $\log y$ vs. $1/T$ curves concave downward (19, 15)—it is realized of course that this is more of a requirement than a proof. Considering (1) Emerson and Arnold (17, Fig. 3, cf. Emerson 14, Fig. 3 and Fleisher 20, Figs. 5 and 8, using a maximum value of (Chl_t) 75% that of Ref. 17) obtained a hyperbolic y vs. (Chl_t) curve, the chlorophyll concentration at half maximum velocity being 1.5×10^{-8} mols per cmm. of cells, but a linear increase in yield per flash with (Chl_t) . It is thus evident from these results, taken literally, that one of three explanations is necessary on the basis of the proposed mechanism, (a) the enzyme is becoming saturated or (b) the amount of enzyme per cell is decreasing, or (c) the variation in y with (Chl_t) is entirely incidental. One would not expect (b) to be the explanation; instead an increase in (E_t) would rather be expected and would tend to hide the concave nature of the curve (Table V). Although test (4) above indicated in two experiments that the Blackman reaction was first order it is doubtful if the chlorophyll concentrations were high enough to be saturating as indicated by figure 3 of reference 17. The second, third, fourth and fifth tests would distinguish between concavity due to variation in enzyme concentration (b) or incidental factors (c) and saturation (a). Certainly some of these tests should

* If the Blackman reaction involved were only (IV) of schema B rather than as pictured in schema A the velocity of photosynthesis at high values of I and (CO_2) should increase linearly with the chlorophyll concentration. It is to be noted also that, (E_t) being held constant, were only a dissociation product of Complex_{II} involved in the enzymic Blackman reaction and not chlorophyll, the downward concavity would not be obtained unless there were inhibition of step (I) or/and (II) by the product or unless unlimited accumulation of such product occurred.

** $\ln(K-M)/K$ vs. time plots should be concave downward approaching a straight line at larger values of t (K = maximum yield, M = yield per flash).

be performed when one obtains positive results by test (1).

Correlation of somewhat indirect facts in terms of the schema includes (1) 3 to 6 fold variation of dark time required to give half maximum yield in flashing light (16), indicating variation in (E_t); (2) smaller scattering of points when flashing light yields are plotted against (Chl_t) (17, Fig. 2), indicating that part of the scattering of the continuous light data (17, Fig. 3) is due to variation in (E_t); (3) 10^{-4} M HCN decreases the rate of photosynthesis in continuous light and increases the dark time in flashing light so that the inhibition is respectively about 60 and 45% (16, p. 407), indicating that the same reaction is being inhibited in both cases; Kohn (29) has also localized the effect of H_2S , iodacetate and aging to the Blackman reaction (aging presumably reducing the (E_t)); the HCN inhibition appears to be competitive since the inhibition decreases from 40 to 25% when the (Chl_t) is increased 2.2 fold (15, Table IV). Inhibition by the "specific narcotic" hydroxyamine (36) should be studied in flashing light. (4) The rate in continuous light can be calculated from the rate in flashing light (17), indicating, in agreement with the mechanism, that the system is not essentially altered when flashing light is used (see also section on induction). (5) On the basis of the dissociation constant 1.5×10^{-2} M Chl_t per cmm. of cells given for (III) 90% saturation of E would occur at about 15×10^{-2} M. (6) The ΔH of (III) might be determined by measuring y as a function of (Chl_t) with high values of I and (CO_2) at two or more temperatures (this of course involves the usual assumption that (III) is essentially at equilibrium).

Temperature. The implications derived in the past (39, 37) from variations in the rate of photosynthesis with temperature demand a detailed analysis of the proposed schema in the light of this variable. Table II, in agreement with experiment (Warburg, 39, Table 4), gives the tem-

perature coefficients under limiting conditions derived from schema A. This combination of temperature coefficients cannot be represented by less than three reactions (cf. (5), (18), (7) and (10a)). If diffusion were limiting at low values of (CO_2) the kinetic equation would reduce to $\log y = -Q'/RT + \text{constant}$ (Eq. 24), where Q' corresponds to the practically negligible temperature coefficient of diffusion (cf. 37).

Emerson and Green (18) have reported data on the photosynthetic activity of *Gigartina harveyana*, *Chlorella vulgaris*, *Chlorella pyrenoidosa* and *Hormidium flaccidum* at high (limiting) values I and (CO_2). These conditions yield $\log y = -\log D + \text{constant}$ (schema A), which only becomes linear in $1/T$ subject to the assumptions in the footnotes of Table II. From the data between 8 and 28°C the value of the Q term may almost be represented as a constant (ca. 12,000 cal) within experimental error. More definite knowledge with regard to the probable error in the temperature range between 8 and 1°C and the degree of saturation with respect to light and CO_2 would allow more definite inference from the observed rapid increase in the temperature coefficient over this small range for *C. vulgaris* and *G. harveyana*. The variations in terms of the mechanism may be due either to I and (CO_2) not being large enough or to the limitations set out in Table II.

Data of the same authors on *C. pyrenoidosa* and *Gigartina* show temperature independence at low values of I and high values of (CO_2), as predicted and in the case of *Gigartina* a smaller temperature coefficient at high values of I and low values of (CO_2), corresponding to Q_1 (6000 to 12,000) in the mechanism (Table II), than with *C. pyrenoidosa*. Their interpretation for *Gigartina* that it seems probable that the lower temperature coefficient at low as compared with high CO_2 concentrations is due in part to diffusion seems undesirable in view of the strict hyperbolic nature of y as a function of (CO_2) (19, figure 4,

TABLE II. $\log y$ as a Function of Temperature Under Limiting Conditions of I and (CO_2)

	High (CO_2)	Low (CO_2)
High I	$-\log D^* + \text{constant}$ [$-\log D^* + \log(\text{Chl}_t)$]	$-Q_1/RT + \text{constant}$ [$-Q_1/RT + \log k_1(\text{CO}_2)(\text{Chl}_t)$]
Low I	constant [$+\log k_3 I(\text{Chl}_t)$]	$(Q_2 - Q_1)/RT + \text{constant}$ [$(Q_2 - Q_1)/RT + \log k_1 k_3 I(\text{CO}_2)(\text{Chl}_t)/k_2$]

* When $y \ll k'_7(E_t)$ (apparently true at ordinary Chl_t concentrations) and $k'_7 \ll k'_8$ (frequently the case with enzymes) $-\log D$ becomes $\log k'_7 k'_8(E_t)/[k'_8(E_t) + k'_8]$ and if $Q_5 = Q_6$ or $k'_8 \ll k'_5(E_t)$ then $-\log D = Q_7/RT + \text{constant}$; or if $k'_8 > k'_5 E_t$ then $-\log D = (Q_6 - Q_5 - Q_7)/RT + \text{constant}$.

TABLE III. Observed Complex-CO₂ Dissociation Constants (K) in Photosynthesis

Investigator	Warburg (39)			Emerson and Arnold (16)		van der Paauw (38)	Emerson and Green (19)	Harder (22a)
Organism	Chlorella pyrenoidosa			Chlorella pyrenoidosa		Hormidium (Pringsheim strain)	Gigartina harveyana	Fontinalis
Condition	high light intensity			continuous light	flashing light	2 x 10 ⁻⁴ M phenylurethane	high light intensity	variable light
T°C.	25	10	5	24.3	24.3 5.9	20	15	22
Relative V _{max}	3.7	2.1	1.0	4.3	1.0			
K x 10 ^{6a}	5.1	4.3	4.4	24	6-10 ^c 5	7.3	1.1	.016% KHCO ₃ ^b

a. K is based on the concentrations of CO₂ in the gas phase, mols per liter (see b) (0.01% CO₂ in gas phase = 4.46 x 10⁻³ mols per litre in gas phase = 3.12 x 10⁻³ mols per liter in liquid phase for 10⁻³/α_{CO₂} = 0.7). Values uncorrected for light intensity (see b).

b. Harder's data, given in % KHCO₃, are corrected to I = 0, the observed values of K at lowest and highest light intensities being 0.026% to 0.20% KHCO₃, respectively.

c. A constant internal (respiration) CO₂ supply factor, which corrects the imposed CO₂ concentrations in contrast and in addition to the usual technical respiration correction (cf. Eq. 18), may be used, and yields a better fit to a hyperbole. The factor is to be added to the CO₂ concentration, and has the reasonable value of about 3 in the investigators' units.

Table III) an alternative is that a different chemical reaction (hence a different temperature coefficient) is limiting (Table II). The difference for the two organisms, which is not exceptionally great, may be due to somewhat incomparable conditions of light and CO₂ or possibly to actual variation in Q₁ with the organism. The sugges-

tion (19, p. 831) that the rate of photosynthesis is a logarithmic function of (CO₂), while true for the experimental range observed, leads to the physico-chemical absurdity of zero velocity at a finite CO₂ concentration.

From the flashing light experiments of Emerson and Arnold (16, Tables IV and V) one may

TABLE IV Observed Complex-CO₂ Dissociation Constants (K) in Photosynthesis, Diffusion Evident

Investigator	Hoover, Johnston and Brackett (24)					van den Honert (37)		van der Paauw (38)	James (25)
Organism	Triticum (Marquis variety)					Hormidium (Honert strain)		Hormidium (Pringsheim strain)	Fontinalis
T°C.	22°					12°	20°	20°	20°
Relative light intensity	1	2.6	4.0	5.5	6.0	3.1	1.0 3.1		
Relative V _{max}	1	2.6	4.1	5.4	6.0	1.1	1.0 1.75	—	—
K x 10 ^{6a} Method B ^b	2.3 1.3 1.0					1.2	1.4 1.2	1.3	2.3
K x 10 ^{6a} Method A ^a	3.2						(0.5)		
" " C ^b	2.0						2.0		

a. See note (a), Table III.

a'. As (a) but corrected to I = 0.

b. See reference (31), p. 664.

TABLE V. Curvature of y vs. (Chl_t) Plot; Theoretical Qualitative Significance (Schema A)

Change of (E_t) per cell as (Chl_t) increases	E inappreciably saturated ($<10\%$)	E appreciably saturated ($>35\%$)
No change	(1) linear	(4) concave downward
Increases	(2) concave upward	(5) concavity $<$ in (4) to concave upward
Decreases	(3) concave downward	(6) concavity $>$ in (4)

get the order of magnitude of $K_{Complex_1} = k'_2/k'_1$ at 24.3° and 5.9°C . The values as determined are respectively $6 - 10 \times 10^{-6} \text{ M}$ and $5 \times 10^{-6} \text{ M}$ so that the ΔH of dissociation by the van't Hoff equation is of the order of 1300 to 6200 cal.*

Baly (6), employing the data of Emerson (15), has reported a fit of a family of curves on photosynthesis as a function of temperature, chlorophyll being the parameter, by an equation of the form, $\log y/(K - y) = \log K_2 - Q/RT$. The significance of the fit, of course, depends on the implications of the equation. A general explicit expression for what appears to be Baly's schema (i.e. reactions (1) and (2) of his paper (p. 224) and the second equation on p. 219, all considered irreversible, and a reaction in the form of reaction (I) of our schema A, which would seem to be implied by his use of the Langmuir adsorption formula) can be reduced to his equation 2 (p. 220), depending on the magnitude of one term. (Equation 2 as derived by Baly shows K as including bA , which may itself be temperature sensitive.)

It is demonstrated in the reduced explicit solution that the first constant term, corresponding to Baly's K , should vary directly with (Chl_t) or the extinction coefficient, which obviously was not done (p. 222). Furthermore, $K/(Chl_t)$ should bear a constant relation to K_2 (K_2 corresponds to k_2c/k_1I in Baly's equation 2). The relative ratios of $K/(Chl_t)$ to K_2 employed, however, were 1, 3.47 and 1.76. Finally the value of K_2 should be independent of (Chl_t) —the variation that Baly has allowed in K_2 would correspond to something like a 2 to 5 fold change in (CO_2) . It is doubtful if Baly's fit of Emerson's family of tempera-

ture curves is of any more than empirical significance, first for the above reasons and second because it is not known to what extent secondary experimental factors are in operation at the higher temperatures. In the great majority of enzyme reactions one must neglect considerable suboptimal portions of the activity-temperature curve in the region of the optimum. Even before the optimum is attained some secondary incidental factors are operating.

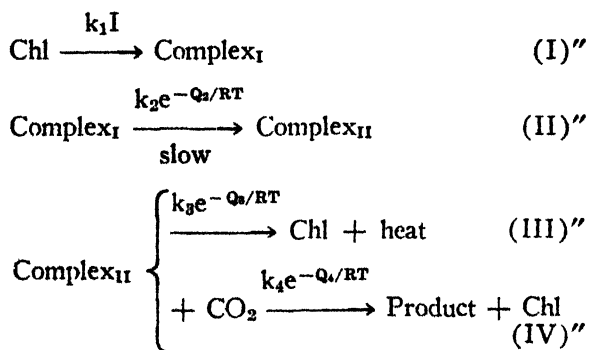
Further consideration of the proposals of Baly (6) exposes the following important considerations. (1) A somewhat artificial and unnecessary method of correlating the flashing light results is used (p. 220). (2) Baly's assumption of the necessity of both red and blue light is not in accord with the fact that photosynthesis takes place quite readily in red light alone, $\lambda = 610 - 690 \text{ m}\mu$, or in yellow light alone, $\lambda = 578 \text{ m}\mu$ (42). He also indicates the use of only two quanta, which is not in accord with the accepted four or minimum three quanta requirement. (3) Carotene and xanthophyll are implicated in the mechanism but the manner in which xanthophyll is reduced to carotene in the cycle is not suggested. It would no doubt be convenient to have the reduction require light. The assumption that the concentration of carotene (p. 220) is large is puzzling in view of the fact that in many plants the total amount of yellow pigments is about 20% of the total chlorophyll and similarly the amount of carotene is about 20 to 25% of the total yellow pigments. (4) The formation of chlorophyll b by Baly's mechanism would suggest that it would be possible to find a shift in the relative amounts of the a and b pigments in the presence and absence of photosynthesis. This has not been shown to be the case. (5) His treatment of poisons is consistent with competitive and non-competitive inhibition theory for enzymes. Table XI, p. 237 of his paper gives an interesting comparative calculation.

Induction and Fluorescence. The quantitative aspect of induction will not be treated here. Qual-

* The order of magnitude of $K_{Complex_1}$ is confirmed in continuous light for several organisms (Tables III and IV). Of definite comparative interest is the heat of dissociation of nitrogen-nitrogenase of $0 \pm 1000 \text{ cal}$ (32); on the other hand, with oxygen-oxygenase, oxygen-hemin compounds, and carbon monoxide-reduced blood, the heats of dissociation are considerable (10, p. 37), 12,000 to 22,000 cal.

itatively, by the building up of intermediate steady state concentrations, the schema would certainly permit an induction period. Without commitment as to the importance in photosynthesis of fluorescence and of monodehydrochlorophyll postulated by Willstätter (44) and Franck (21) it should be pointed out that schema A may harmonize, by addition, with such considerations. The duration of induction, 1 to 2 minutes, may bear some relation to the fluorescence studied by Kautsky and co-workers (26, 27, 28). It would be desirable to extend the temperature HCN, indifferent narcotic, photosynthesis quotient and even flashing light studies of induction and fluorescence to determine whether a step or steps in schema A, or some additional reaction is responsible. The facts that there is no induction at low light intensity (40) and that induction has a temperature coefficient (van der Paauw 38, $Q_{10} = 2.2$ for *Hormidium*) suggest that it has to do with a dark reaction. Such a dark reaction of course would of necessity occur after the light step (II). The requirement of five minutes to return to dark equilibrium (40) (which incidentally tends to validate the extrapolation of flashing light results, where dark times of the order of hundredths of seconds were used after an appreciable adjustment period, to continuous light data) points to the conclusion that the induction is controlled by an additional reaction or reactions since evidently the data from which schema A was deduced were obtained after time had removed this phenomenon.

Schemata Involving CO₂ after the Light Reaction and E before the Light Reaction. It is possible to place CO₂ after the light reaction with respect to a given molecule or unit of CO₂ but certain apparently necessary deductions make such schemata at present less desirable considerations. The simplest schema designated as schema C, may be represented as follows:

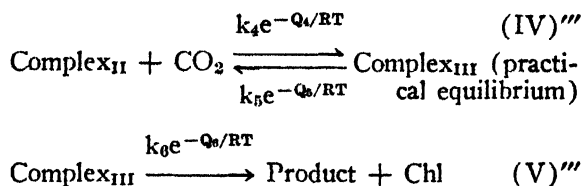


The kinetic equation derived as before is:

$$y = \frac{k_1 k_4 k_2 (\text{Chl})_t I (\text{CO}_2)}{k_1 k_4 I (\text{CO}_2) e^{Q_4/RT} + k_2 k_4 (\text{CO}_2) + I k_1 e^{Q_4/RT} (k_3 e^{(Q_3-Q_4)/RT} + k_2) + k_2 k_3 e^{(Q_1-Q_3)/RT}} \quad 31$$

which is in exactly the same form as Eq. 14, when the factor D is constant (Eq. 13), with the exception of the temperature term at high values of I and low values of (CO₂). The following considerations would seem to be sufficient to make this mechanism untenable: (1) In order to explain the fact that the yield per flash at high values of I and (CO₂) is independent of temperature it is necessary to assume that (III)'' and (IV)'' have the same temperature coefficient. (2) In order to explain the independence of the dark time on (CO₂) (16, p. 411) it is necessary to assume that (III)'' is much faster than (IV)''. This requires however that the yield be proportional to (CO₂), which is not the case (16, p. 411), and it does not allow a high efficiency at low values of I.

A schema D may be described in which reaction (IV)'' is replaced by the two reactions



The kinetic equation will be essentially the same as Eq. 31 for continuous light. The deductions for flashing light would remove objection (2) above but not (1).

UNITY OF CARBON DIOXIDE REDUCTION IN NATURE

It appears to us very possible that throughout Nature carbon dioxide is reduced chemically, not photochemically, from the standpoint of mechanism. We would suggest that in Nature a fundamental mechanism has been established which operates in an essentially uniform (non-photochemical) manner throughout the three major classes of autotrophic organisms: 1, the chemosynthetic bacteria, which employ chemical energy only in the overall process of reduction; 2, the photosynthetic (or, better, photo-chemosynthetic) bacteria, which ultimately employ both chemical and radiant energy simultaneously; and 3, the green plants, which ultimately employ radiant energy only, in the overall process. In all cases CO₂ may combine chemically with some substance (or catalyst) and be reduced; the substance which it has oxidized is then reduced back to its original state: respectively, 1, chemically; 2, chemically and photochemically; and 3, photochemically. By this conception, the proposed

common autotrophic mechanism for reducing CO_2 (as to a stage approximately $(\text{CH}_2\text{O})_n$) might well have been originally established, from the evolutionary view, in the strictly chemosynthetic organisms (class 1). Later, after many organic compounds had been evolved, including pigments capable of utilizing radiant energy, the autotrophic mechanism would then have been augmented, in classes 2 and 3, by a photosynthetic mechanism capable of reducing, not CO_2 , but some product which CO_2 has oxidized in becoming reduced.

This simple, evolutionary outlook is not formal; it indicates that, in Schema A, *reduced carbon* may appear—to some (small) extent—as an addition product in reaction (I), before the light reaction has taken place. Some additional reducing component may be involved in reaction (I), indicable either as an additional reactant or reaction, but in any case the overall formation of Complex_1 must of course be reversible, and capable of proceeding only so far, until supplied further with energy by reaction (II), in the sense that reaction (II) removes a product of reaction (I), the substance oxidized by CO_2 . The mechanism of Conant, Dietz and Kamerling (13) provides for a chemical reduction of CO_2 specifically by chlorophyll, in higher green plants, and is based on certain chemical evidence. It bears only a certain resemblance to the more general view advocated here, which is applied to *all* autotrophs.

SUMMARY

A schema for photosynthesis has been presented which should enable general thinking to adopt particular representations. The schema or minimum mechanism consists of a composite of light reactions grouped kinetically into one light, and three essential dark, reactions; the group of light reactions represents neither the first nor the last but rather the second reaction with respect to a given CO_2 molecule. The schema has been deduced from photosynthetic data concerned with the following considerations, often studied under several combinations of limiting conditions: the carbon dioxide and chlorophyll concentration functions; the light intensity function; the temperature coefficients; flashing light experiments that involve orders of reaction and dependence of maximum yield and of time required for half completion of the dark reaction on temperature, CO_2 concentration, chlorophyll concentration, HCN, etc.; the effect of indifferent and specific narcotics in both continuous and flashing light experiments; the characteristics of the Blackman reaction; and diffusion.

The schema suggested by these factors has in turn suggested other important lines of attack. The following have been considered: (1) method

and desirability of analytic analysis of light and CO_2 data and approximations involved, (2) the criteria of significance to be used when CO_2 uptake is measured in the absence of photosynthesis, (3) the use of indifferent narcotics to test the possibility of Blackman enzyme saturation at high chlorophyll concentrations, (4) confirmation of enzyme saturation by accurate determination of the order of the dark reaction as well as the maximum yield per flash at high chlorophyll concentrations, (5) further confirmation by comparing the survival ratios with regard to ultraviolet light treatment in continuous and flashing light as a function of chlorophyll concentration, (6) separation of the composite light reactions (e.g. by decreasing the length of the light flash), (7) repetition of certain work with adequate determination of the statistical probable error and potential consistent error as well as with proper regard for and determination of critical ranges of variables and critical experiments.

The following considerations are believed to be new either in proof or deduction: (1) the reversibility and reaction order of step (I) demonstrated by flashing light data and linear plots, (2) calculation of the order of magnitude of the dissociation constant (5×10^{-6} M CO_2 in the gas phase) and ΔH (4000 cal.) of step (I), (3) the separation of the Blackman reaction into two parts based on the non-linear increase in y as a function of chlorophyll concentration (apparent saturation of an enzyme), (4) the magnitude of the dissociation constant of step (III), 1.5×10^{-8} mols per cmm. of cells, (5) competitive inhibition by HCN in steps (III) and (IV), and (6) certain parts of the kinetic treatment such as indicating four possible temperature coefficients at limiting values of I and (CO_2).

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DISCUSSION

Dr. Briggs: The very condensed form in which the suggestions in this paper* are given renders discussion difficult. Perhaps in the following comments I may be guilty of misconstruing their meaning. The general form of the schema is the same as that suggested by myself (2) except that a hypothetical "substance S, which may be chlorophyll" was assumed to combine with carbon dioxide. In that paper the schema was used to explain induction phases. In a paper (3), now in press, the schema has been

* i. e. 10a.

used to interpret the results of experiments with intermittent illumination. There it is shown that experiments with constant illumination are in general agreement with the schema, but stress is laid on the complications in most assimilatory systems—heterogeneity of illumination, interaction of respiration and assimilation, etc.,—which render interpretation of the results difficult. The development of the equations differs from that by Burk and Lineweaver. Firstly it is not assumed that the reverse reaction in II can be neglected. The reasons for this are given in detail in the paper referred to above (3). Since with high intensities of illumination only a small fraction of the energy absorbed is utilized in assimilation, much must be lost. In the schema suggested the reverse of II is the only stage where loss can occur. Secondly, allowance is made for the fact that D, in equation A, is a function of y . I assume that "the Blackman reaction is apparent first order" means first order as regards activated complex of chlorophyll and CO_2 and refers to the argument of Arnold (1) from the fact that exposure to ultraviolet light depresses the rate of assimilation in bright light to the same extent whether the latter is continuous or flashing. As I have pointed out (3) this result is open to other interpretations than the one that practically the whole of the catalyst E is in the free form. Only with these simplifying assumptions is equation A explicit in y . Without them y is not proportional to $\text{CHl}_{\text{total}}$. The fact that in general there is this lack of proportionality suggests that the simplifying assumptions are not justified.

Ref. 12 in the paper of Burk and Lineweaver assumes that variation of yield per flash in flashing light with variation of length of dark period is a measure of the dark period reaction. In the above paper (3) it is shown that the assumption is justifiable only when the concentration of carbon dioxide is so great that re-formation of the complex is complete however short the dark period and when the flash is so intense that the whole of the complex is activated, otherwise the amount of activated complex will depend upon the amount left at the end of the dark period.

Dr. James: It is perhaps a weakness of the proposed theory that it assumes (in common with other such theories) that the rate of the photo-reaction itself is proportional to "light intensity". The term is not closely defined in the published paper* and so we cannot be sure of the author's concept. Experimentally light intensity is usually measured in terms of energy incident on a surface at or near the surface of the photo-

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synthesising organ. Apart from difficulties due to the different qualities of the energies measured and those photosynthetically active, the rate of the photoreaction will only be proportional to the intensity of the effective incident energy ("light") under restricted conditions, affecting both the physical and chemical aspects of the system. The nature of these conditions I have endeavored to set out in another place (*New Phytol.*, 33, 8, 1934). They are rather stringent, and leaves with a complex distribution of chloroplasts, or a deep algal suspension (the systems most frequently used in this type of work), depart rather widely from the physical requirements. On the chemical side, since there is little probability of the reaction having a unit quantum efficiency, further assumptions are necessary that do not seem to be provided for in the suggested scheme. Possible alternatives are a series of photoreactions, a photoreaction involving the simultaneous impact of more than one quantum (such a possibility does not seem to have been disproved for so complex a molecule), or gain of energy in a thermal reaction. The fact that qualitative and occasionally semiquantitative predictions of results can be made with the (apparently) simple relationship suggested should not blind us to the theoretical complexities ignored. We might thus be blocking the road rather than smoothing it for further progress.

The term "carbon dioxide concentration" is also somewhat ambiguous. It seems to be assumed that the "incidental physical diffusion of carbon dioxide" may be neglected in predictions of rate, and that (CO_2) may stand indifferently for the measurable external concentration and concentration at the chloroplast surface. This is far from the truth at moderate concentrations. I have been able to show that considerable variations of rate result from adjustments of the diffusion path (*Proc. Roy. Soc., B*, 103, 1, 1928), and the very elegant experiments of van den Honert show that the short passage through the cell wall, etc., to the chloroplast (estimated at a few $m\mu$) has well marked effects. The use of bicarbonate solutions to evade this difficulty is objectionable since they cause observable depression of photosynthetic rates even at low concentrations (*New Phytol.*, 1934). A general kinetic scheme, as distinct from one applying only to special and restricted cases, cannot, therefore, afford to ignore this term. An improvement is made by the adoption of even so simple an expression as

$$y = r + [(\text{CO}_2)_e - (\text{CO}_2)_i]/d$$

where e and i indicate "external" and "chloroplast surface" concentrations respectively; d a resistance term which is large since we are dealing

with hydrodiffusion, and r the respiratory contribution of carbon dioxide which may perhaps be neglected. Since the carbon dioxide has to pass not only through a simple water layer but also through an approximately semi-permeable membrane, the value of d above may be considerably greater than that of the hydrodiffusion constant for carbon dioxide.

It is a formal necessity of the equations that the Blackman reaction should be regarded as proceeding in two steps; otherwise obvious discrepancies arise between the theoretical predictions and observed results. If the reaction is enzymically catalysed two steps are highly probable on general grounds. So far this reaction has been subjected to but little direct study, and it is, therefore, with great interest that I learn that Burk and Lineweaver consider that "It consists observably of two consecutive reactions." The publication of the full details as promised should do much to strengthen the position of theories such as theirs and my own at one of the most speculative points.

Dr. Boysen Jensen: It is with great interest that I have read the note in *Nature* by Burk and Lineweaver on the kinetic mechanism of photosynthesis. I feel that I am not quite competent to discuss the problem, as my own investigations on photosynthesis deal with the ecological aspect of this process, and have been carried out with leaves of higher plants; undoubtedly lower plants will fit much better for kinetic studies.

I agree that the four reactions, I-IV, are rather probable, but I think that it is too early to set up a kinetic equation with a series of constants that we are not able to estimate, wherefore we cannot check the correctness of the equation.

Some 25 years ago, physical chemistry was the great hope of physiology, but in my opinion the great expectations in regard to this branch of science have only to a very small degree been fulfilled. For instance, as to the kinetics of enzymes, we have a large number of papers that nobody reads. The great progress in physiology has not been attained by setting up "laws", but through a more quantitative study of reactions in the living organism, through attempts to divide complex reactions into simple ones with isolation of intermediate products, and through discovery of physiologically active substances or hormones and vitamins; in my opinion organic chemistry has yielded more to physiology than has physical chemistry.

It seems to me that the most promising way, in analyzing the mechanism of photosynthesis, must be to secure better experimental evidence for the four reactions than we now possess, but I think it will be difficult. It will be claimed that the equations developed should be an aid to obtain a

better insight, and should that be the case, I shall of course receive every progress with delight.

Dr. Burk and Mr. Lineweaver: It must be mentioned that Dr. Briggs, Dr. James, and Dr. Boysen Jensen were provided only with our short note in *Nature*^(10a). Perusal of our longer text, and our discussion in Brackett's paper, will show that we are in quite general agreement with their comments, and that we have treated or allowed for most, or all, of the very clearly expressed suggestions they have offered in regard to variable light intensity, variable diffusion resistance, respiration carbon dioxide as affecting substrate concentration, four-quanta requirement, explicitness of γ (D being the determining factor), and estimation of constants. The first three factors we are inclined to regard as incidental to an eventually determined chemical mechanism (in the sense of Rollefson, below), and to be avoided experimentally wherever possible, or where this will often be impossible, corrected for analytically by the simplest possible assumptions. The following differences of opinion or outlook may now be noted, in regard to the criticisms submitted.

We should not like to imply that the hypothetical "substance S (which may be chlorophyll)" employed by Briggs results in any more than a difference in nomenclature (we have been a bit more specific in regard to a definite physico-chemical schema). However, we do not have a feeling for the "novel" inhibitor mechanism. Reaction (II) need not be reversible, as stated by Briggs, since a low efficiency at even high CO_2 may be obtained if light is degenerated by being absorbed by complex II, which can presumably undergo no further photochemical reaction. As complex I becomes (irreversibly) converted to complex II, the efficiency must necessarily decrease, since the only light absorber capable of converting the radiant energy into chemical energy is then being depleted. Furthermore, if reaction (II) were reversible, it would seem difficult to explain the high efficiency at low I since the fraction of complex II lost to the photosynthetic cycle should be independent of the concentration, other factors being held constant.

The evidence we employed in favor of an apparent first order Blackman reaction, given in the text, is not based on Arnold's data, which is, we agree, easily open to the other interpretations. We are also in complete agreement with Briggs' final paragraph.

Although, as James indicates, it is *a priori* probably a formal necessity that the Blackman reaction be written in two steps, our treatment attempts to show to what extent actual saturation of the enzyme is involved, as distinguished from mere amount limitation, which has long been highly probable experimentally. As James points

out, the emphasis of interest lies on the "observability" of the saturation (capacity limitation).

Boysen Jensen will no doubt be interested to note the large number of constants which have been estimated for reactions I-IV in the case of the explicit solution of Mechanism A (D constant, not a function of γ). All constants for Harder's data, in terms of Chl, which itself might also have been measured, are reported. The accuracy of the determinations of the constants would of course have to be greatly improved with much more extensive data; Harder's data were used only for the purpose of illustration. In regard to the remarks on the influence of physical chemistry in physiology, with which we agree of course, Harris (23) has recently pointed out similarly that genetics "upon coming of age has become more and more qualitative" and that it cannot do so without having serious repercussion in the whole science of biology. In our opinion, however, the problem of photosynthesis is essentially too non-biological to share either of the fates indicated by Boysen Jensen or Harris, but will tend progressively to become more and more quantitative, as commented upon in our Introduction. In other words, we are inclined to believe that the major interest in photosynthesis has already turned from physiology, is perhaps now chiefly concerned with physico-chemical aspects, and will probably eventually be of particular interest in physics, for which it now presents a unique photochemical problem, namely, an endothermic reaction requiring several quanta.

Dr. Rollefson: The "mechanism" presented in the paper is not a mechanism in the same sense that that term was used during the first week of this symposium, inasmuch as no attempt is made to specify the chemical nature of the molecules involved in every step. The four relationships which have been given may be thought of as representing the result obtained by dissecting the rate equation into parts and writing a generalized type of chemical equation to conform to each part. These parts are then arranged in a sequence which permits the explanation of facts other than rates. Such a procedure gives a framework which is useful in setting up mechanisms using specific chemical molecules.

Dr. Burk: I am glad to have this point brought up. In the text it is only briefly indicated in an explicit manner, although to a large extent implicitly. We are definitely limiting our treatment of the problem, in this paper, to what we called in the introduction a "physico-chemical" mechanism, as distinguished from a mechanism involving all chemical aspects. We have set down a "kinetic" mechanism with which, assuming its correctness, established and to-be-established facts of a more generally chemical nature (specific chemical molecules) will presumably have to be

consistent. At present there are relatively more facts known about the kinetics of photosynthesis than about most of its other aspects, and this accounts, in part, for our point of approach.

Dr. Rollefson: I would like to point out that derivation of the correct rate law from a mechanism is not sufficient to prove the mechanism correct. It is often possible, even in relatively simple systems, to set up a number of mechanisms which will give the correct rate law, and the decision between these must be made on the basis of other observations such as the chemical properties of the assumed intermediates.

Dr. Burk: Yes. We have remarked elsewhere (32), in a discussion of the same treatment applied to enzymes, or to homogeneous or heterogeneous catalysis generally, that detailed analyses of kinetic data determine what mechanisms *may*, but not *necessarily do*, hold, and in particular eliminate certain mechanisms which definitely *do not* hold. Non-conformity of a set of data with a given mechanism eliminates that mechanism unless closer analysis indicates that some consistent experimental error might be involved. Needless to say, all due caution is necessary in concluding that a reaction follows a certain mechanism because a set of data fits a certain equation. Lack of extensive data might also be responsible for a misleading agreement of data with an assigned mechanism. The direction of purposeful experimentation is in any case the more evident.

Dr. Rollefson: Your procedure may be looked upon as a particular solution, and not as a general solution which is capable of excluding all "physico-chemical" mechanisms which do not fit this form.

Dr. Burk: For several years we adopted the view that we were dealing with a particular solution, but during the past year we have come to believe that without inserting a large number of additional *ad hoc* reactions, our minimum mechanism is reasonably unique, except for certain qualifications given in the text, and that it excludes all other mechanisms inconsistent with it, at least so far as the plants, data, and accuracy of data involved are concerned. We have compared it with all the many and known major mechanisms proposed heretofore, and also with many arbitrary ones set up by ourselves. But apart from this process of elimination, it must not be forgotten that there is a fit of several score qualitatively different facts, consistent with our best chemical experience, and that under this condition the number of possible fits, or types of fits, is in any case reduced to two or three at most. For the problems of orders and sequence involved, the answer is highly determinate. We are, therefore, inclined to regard our mechanism as being not far from a general solution for the known physico-chemical aspects, barring a detail

here and there. Our expectation is that each of the four reactions given will eventually be consistently expanded into two or more sub-reactions which it will be possible to observe analytically under properly arranged experimental conditions.

Dr. van Niel: If the number of light reactions is, let us say, 2, 3, or 4, is it not possible that your system of equations becomes very different from the system you actually write?

Dr. Burk: No, at least not necessarily. The whole, overall light reaction is very fast, but one reaction may be much slower than any of the others, in harmony with the observation that in continuous light the light intensity never enters to more than the first power (order).

Dr. Mestre: Do I understand that in Reaction (II) you postulate that there are four continuous light absorptions without any dark reactions occurring in between?

Dr. Burk: No, they may occur, but they must be of the right (sufficiently high) velocities.

Dr. Brackett: Is the location of the enzyme consideration with respect to the limiting light reaction, or to all photochemical reactions?

Dr. Burk: To all of them presumably. This limitation does not arise, of course, until a considerable concentration of Complex II is built up, which means high I at high Chl_a and at least moderate (CO_2).

Dr. Brackett: If there were a light reaction subsequent to the slowest light reaction, in which the dissociation of the products were limiting, wouldn't that exert an influence similar to that of the postulated enzyme?

Dr. Burk: No, not if we accept the flashing light experiments showing independence of yield in the time of the light flash.

Dr. Brackett: Is there a tacit assumption that unless the chlorophyll concentration is varied by some artificial means it remains constant throughout an experiment?

Dr. Burk: Yes.

Dr. Brackett: We have observed such variations in apparent chlorophyll concentrations under varying conditions of illumination. Where points are taken rather slowly over considerable lapses of time, even several hours, it may be conceivable that the experiment is not dealing with the same amount or condition of chlorophyll during the whole experiment.

Dr. Burk: Yes that is conceivable, but is presumably a negligible consideration for most of the very short time experiments we have in general considered. It undoubtedly represents one of the innumerable factors occasionally operating unbeknownst in photosynthetic experiments.

Dr. Curry: Professor Trelease and I are experimenting at Columbia University with photo-

synthesis in *Chlorella*, using heavy water (Science, 82, 18, 1935). We find that the rate of photosynthesis is 0.4 that in ordinary water. It would seem that the difference in rate cannot be accounted for by mere diffusion. We are still working, using pure heavy water under different conditions of light intensity and CO_2 concentration.

Dr. Burk: The mechanism and methods illustrated in our paper should be of assistance in deciding in what reaction or reactions the heavy water as well as the ordinary water enters. Thus, it may influence the apparent dissociation constant in reaction I, as narcotics or competitive substrates do, or it may compete or unite with E in reaction III-IV, as cyanide does, etc. If D_2O is without narcotic effect and yet gives a different rate, photosynthetic activity as a function of H_2O as a substrate may be determined by varying the ratio of H_2O and D_2O . Comparative experiments with flashing light and continuous light, under various limiting conditions, would be very advantageous, perhaps really necessary.

Dr. Emerson: If your mechanism is so broadly defined that it covers all the different carbon dioxide functions shown in your slide, then I think it must include terms which are descriptive of processes outside rather than inside the cell. At present we must suppose that the assimilatory process itself is always the same function of carbon dioxide concentration, though the experiments available do not permit us to decide with certainty just what the function is.

Dr. Burk: Our main, four-step mechanism refers to reactions located inside the cell, only. By adding an incidental fifth step, allowing for diffusion which may sometimes be involved, the mechanism then takes care of carbon dioxide functions possibly involved outside the cell. The treatment for the fifth step may, if necessary, vary, but so far the simplest possibility appears to suffice to cover most cases. The results of our

analysis of published carbon dioxide functions, whether diffusion is involved or not (five or four steps) all agree in indicating a fundamental hyperbolic function with the constant of dissociation not greatly different among different plants. The evidence is supplied in the table in the text. The function is sometimes altered by incidental factors which may reasonably be removed by analysis.

Dr. Emerson: It should be pointed out that most of the studies on photosynthesis at low concentrations of carbon dioxide have been made in carbonate-bicarbonate mixtures. We do not know whether data obtained in this way are comparable with determinations in less alkaline media, and there is no doubt that some cells are irreversibly injured by the carbonate mixtures, although for *Chlorella* they appear to be quite harmless. But even for *Chlorella*, we are not yet certain that when we are dealing with low concentrations of carbon dioxide the rate of assimilation measured in an alkaline carbonate mixture is the same as would be found in a more physiological medium containing the same concentration of carbon dioxide.

You have attributed great importance to the effect of cyanide on the dark-time curves of *Chlorella pyrenoidosa*. You may be interested to know that the dark-time curves for *Chlorella vulgaris* do not show the same behaviour in cyanide. The inhibition does not vanish with increasing darktime.

Dr. Burk: This may be due to an added, incidental effect of cyanide. The experimental observations that the yield in flashing light is independent of temperature and of cyanide (at high CO_2 and sufficient dark periods) with *C. pyrenoidosa* do seem to us extremely important because of their bearing on whether light reacts before or after the entrance of CO_2 . They should be repeated for many organisms. If not immediately confirmed a closer search for incidental disturbing factors is warranted.

THE EVOLUTION OF OXYGEN IN THE PROCESS OF PHOTOSYNTHESIS

O. L. INMAN

Joseph Priestley (1) placed sprigs of mint under a belljar containing an atmosphere which had become laden with carbon dioxide from animal respiration, and showed that the air lost a large part of its carbon dioxide and gained "dephlogisticated air" or oxygen. Closely following this demonstration, Ingen-housz (2) showed that green plants evolved oxygen only when in light. Soon Senebier (3), Theodore de Saussure (4), Boussingault (5), and later Dutrochet (6) announced that carbon dioxide was absorbed and oxygen evolved only by the green parts of the plants, and that the volume of the carbon dioxide absorbed was equal to the volume of the oxygen evolved. (The process of respiration was neglected in this work.) Bonnier and Mangin (7) used four methods in studying the ratio of carbon dioxide absorbed to the oxygen evolved or the "photosynthetic quotient." They expressed their results in the form of O_2/CO_2 and found a quotient greater than unity (1.05-1.3). Maquenne and Demoussy (8) gave the volume of carbon dioxide absorbed as equal to the volume of oxygen evolved in twenty-nine of thirty-four plants studied. Willstätter and Stoll (9), using a different method, reported a photosynthetic quotient (stated as CO_2/O_2) as exactly one. Kostytschew (10) has brought out the fact that the CO_2/O_2 ratio varies with time. He found that in the beginning of photosynthesis much more carbon dioxide was absorbed from a 6% concentration than oxygen was evolved. After a continued illumination, the relationship was reversed since, over longer periods of time, the ratio CO_2/O_2 was unity.

Knowledge concerning the photosynthetic quotient is valuable, since this relationship should give some evidence as to the oxidation-reduction balance within the plant cell, and thus throw light on the possible chemical nature of the compounds produced and the energy exchanges taking place in the mechanism of photosynthesis. While many refined methods for measuring more accurately the gases absorbed and those evolved have been introduced, the progress made in this study since the time of Boussingault has not been very marked. There yet remains a definite demand for a clearer understanding of the inter-relationship of the processes of respiration and photosynthesis.

The evolution of oxygen in vitro

In 1901, Friedel (11) reported successful extraction of a yellowish substance from leaves which, when added to green leaf powder dried at $100^\circ C.$, would absorb CO_2 and evolve O_2 .

Harroy (12) repeated this work and could not confirm Friedel's results. Usher and Priestley (13) described a method, using extracted chlorophyll with and without sheep liver catalase and a pure culture of Beijerinck's luminous bacteria, which demonstrated that oxygen was evolved more readily from the chlorophyll films on gelatin when exposed to light in the absence of catalase. They felt that their experiments supported the view that extracted chlorophyll solutions when exposed to light would give off free oxygen. They also believed that the oxygen liberated was derived from hydrogen peroxide. Ewart (14), Euler (15), Warner (16), and Inman (17) failed to confirm Usher and Priestley's findings on the evolution of oxygen *in vitro*.

At the present time most of the evidence supports the view that oxygen is not evolved consistently, as in the natural photosynthetic process, in any artificially constructed cell model where the chlorophylls have been extracted from the plant by organic solvents and then exposed to light. Even in the case of the green and purple sulfur bacteria, the fixation of carbon compounds under the influence of visible radiation is not accompanied by the evolution of O_2 . The failure to liberate free oxygen consistently over long periods of illumination, as in the normal photosynthetic process of the green plant, holds true for all living and dead cells that do not possess unchanged chlorophylls. (Unchanged chlorophylls are here defined as chlorophylls with typical absorption spectra, good phase test, and a normal basicity reaction.) When the etiolated plant is exposed to light, the formation of chlorophylls begins almost at once if oxygen, medium light intensity, and favorable growing temperature are present. In the course of about two and one-half hours, the plant begins to show the presence of chlorophylls to the eye and the evolution of oxygen can be detected upon illumination. While these facts indicate the necessity of chlorophylls for the freeing of oxygen by the plant, it must not be assumed that any cell or cell contents possessing unchanged chlorophylls necessarily evolve oxygen upon radiation. There are other obligatory factors concerned in the normal mechanism of carbon assimilation by the green plant.

The evolution of oxygen in the absence of the living cell

Molisch (18), using Beijerinck's luminous bacteria as indicators for free O_2 , concluded from his study of dried and heated leaves of many plants that cells which were not dried above

35° C. would retain the power to evolve oxygen for several days. In some cases he heated the dried leaves to 70 or 80° C. and was still able to detect the evolution of oxygen. His conclusion was that these dried leaves were dead and that, consequently, carbon dioxide could be absorbed and oxygen evolved by dead cells. Several workers criticized Molisch's conclusions, but little actual data was presented to support such criticism. Inman (mostly unpublished data) has studied the evolution of oxygen from the green plant using luminous bacteria as Molisch did. Unless otherwise stated, the data given below are the result of the use of fresh ground leaves of *Trifolium repens*, *Zea mays*, and *Melilotus alba*. The leaves were ground with sand and all cells filtered out, the suspension of chloroplasts and pieces of chloroplasts and other cell constituents being used as the material from which the evolution of oxygen was studied. The luminous bacteria were mixed directly with the cell contents and the inactivation studied by measuring the time when the plant material no longer gave off sufficient oxygen to induce light in the bacteria.

By use of this technique, many kinds of leaves have been dried and tested for the evolution of oxygen. Such leaves as *Trifolium repens*, *Melilotus alba*, *Helianthus annuus*, *Althea rosea* will stand several days of drying at 30-35° C. and still evolve oxygen when allowed to stand in tap water for several minutes before mixing with the bacteria. In the case of *Nostoc sp.* one and one-half years at room temperature has not been sufficient to inactivate the alga. While these results fully confirm Molisch's work, it should be reported that grinding the fresh leaves thoroughly with sand and then drying the macerated tissue or drying the whole leaves and then powdering them causes much more rapid inactivation. A very logical assumption here is that exposure of a larger surface to the free oxygen of the air is responsible for the destruction of the power to evolve oxygen. Another fact to be noted is that fresh green leaves or seedlings of *Zea mays* or *Melilotus alba*, stored under nitrogen, lose their power to evolve oxygen much more rapidly than when they are stored under air, air with 95% oxygen, or carbon dioxide. Examination of the chlorophylls from these leaves inactivated by storage under nitrogen revealed the fact that the chlorophylls had been changed so that either the absorption spectrum, the phase test, the basicity test, or more than one of these, was abnormal. Work on the radiation of pure chlorophylls and some of their decomposition products under pure nitrogen, reported in this volume by H. V. Knorr, shows that a nitrogen atmosphere is very conducive to rapid photo-decomposition of these compounds. In the case

where the ground dried leaves become inactivated before the whole dried leaves, it is not the chlorophylls that are changed but some other factor which brings about the inactivation.

a. *The effect of temperature upon the evolution of oxygen.*

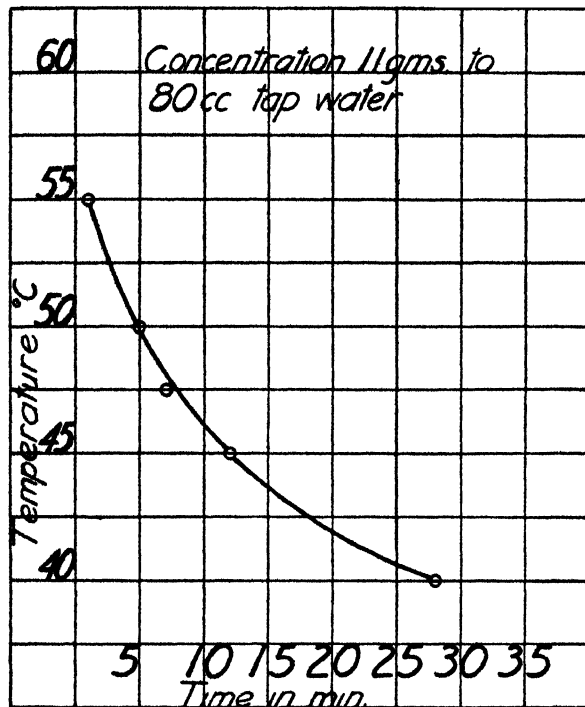


Figure 1 shows the effect of varying temperatures on decreasing the power of the cell contents to evolve oxygen when illuminated.

It will be noted from figure 1 that the effect of temperature as an inactivating agent in water solution shows the maximum at about 55° C., which is not far from the thermal death point for most unprotected cells. On the other hand, it was found that freezing and thawing the cell contents for 57 times with carbon dioxide snow had no detectable effect upon the evolution of oxygen. The exposure of the cell contents to liquid air for 30 minutes brought about no apparent inactivation. In fact, storage of the cell contents at low temperatures demonstrated that the lower the temperature, the longer the power to evolve oxygen remained.

b. *The effect of concentration of the cell contents on the evolution of oxygen*

It is easily observed that figure 2 indicates that any change in the concentration of the cell contents gives a marked change in the inactivation time and that the more dilute the solution, the nearer the approach to a straight line.

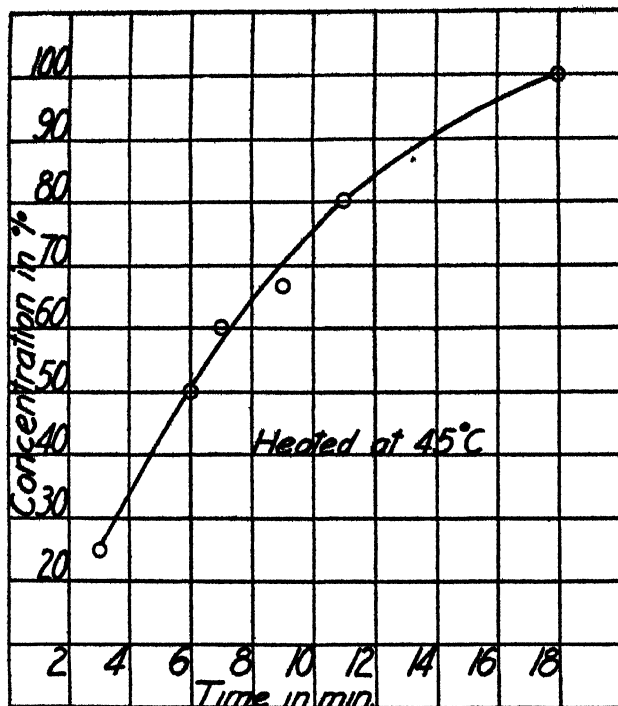


Figure 2 shows the effect of varying the concentration of the cell contents on the time of inactivation of the process of the evolution of oxygen 11 grams of *Trifolium repens* leaves to 80 cc tap water was taken as 100%

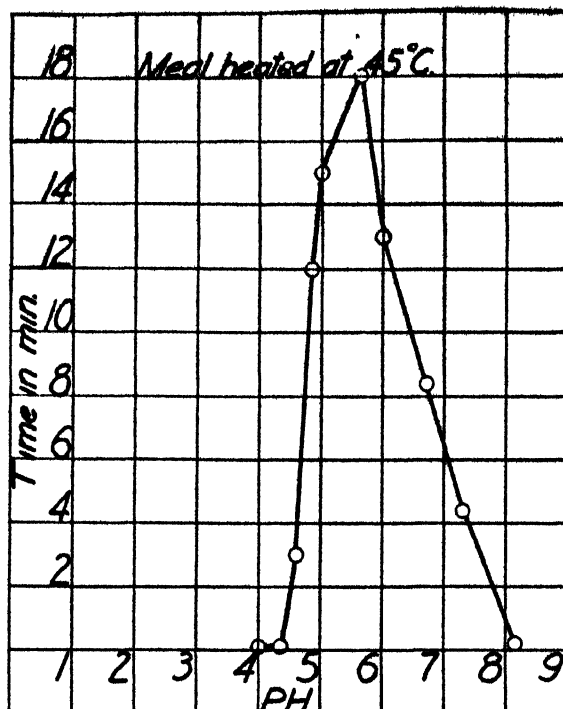


FIGURE 4

Figures 3 and 4 show the effect of pH on the time of inactivation of the process of the evolution of oxygen at definite temperature and concentration of the cell contents *Trifolium repens* was used

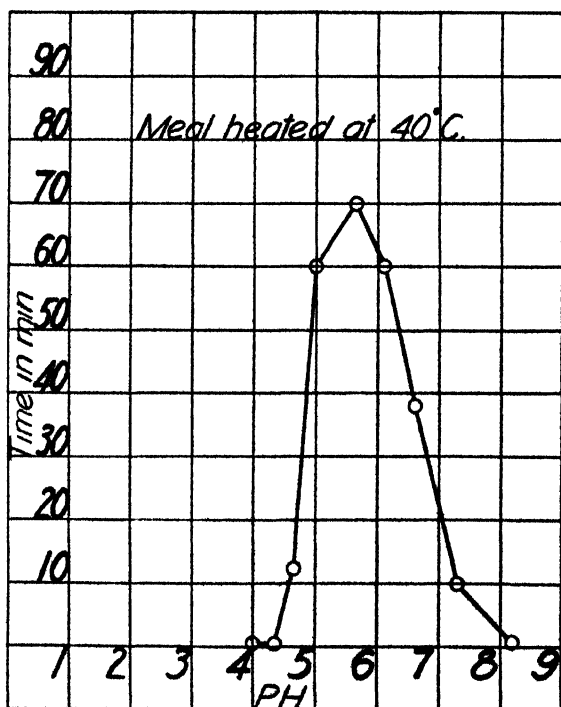


FIGURE 3

c The effect of pH on the evolution of oxygen¹

In a rough way, the curves of figures 3 and 4 demonstrate that the evolution of oxygen by the cell contents depends on the hydrogen-ion concentration and gives a set of curves which approximate a typical enzyme hydrogen-ion concentration activity curve.

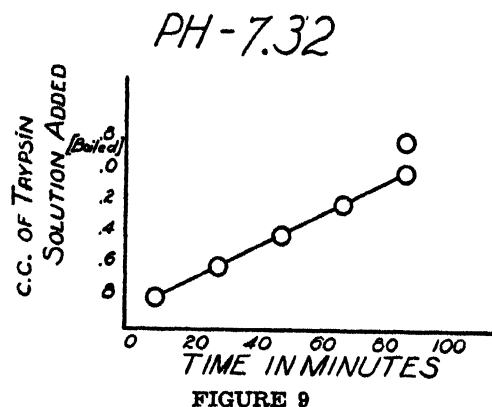
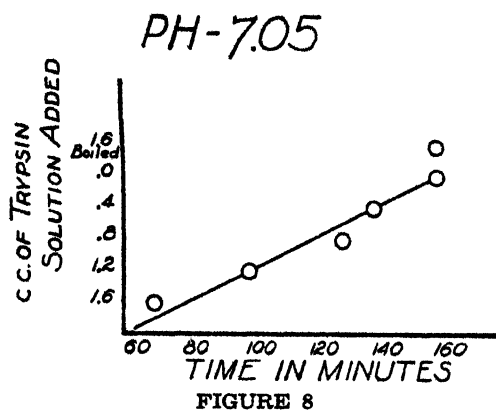
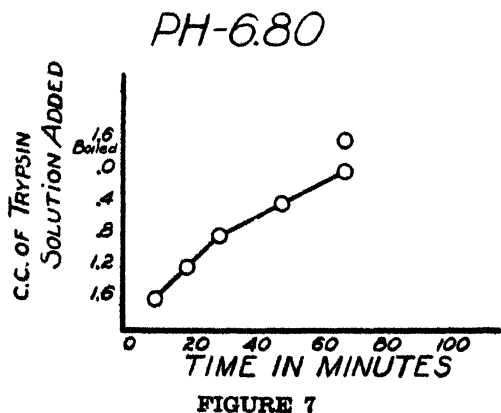
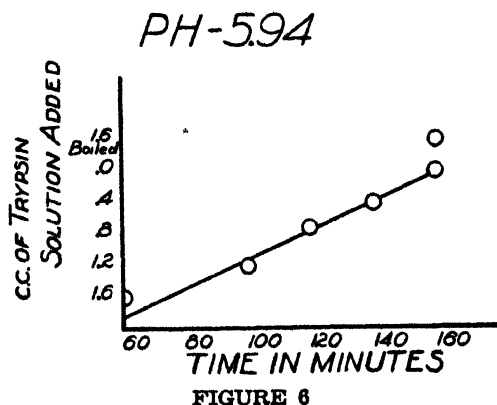
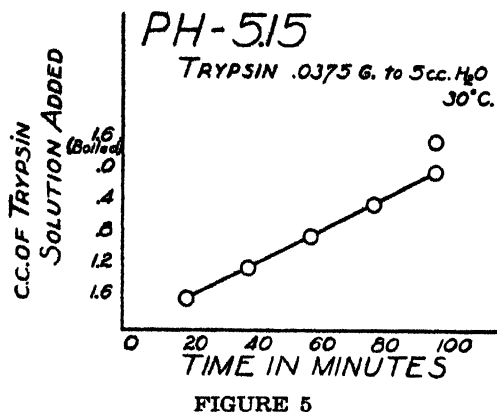
d The effect of enzymes on the evolution of oxygen²

Figures 5, 6, 7, 8, and 9 show that the amount of inactivation is almost directly correlated with the amount of enzyme added to the solution. This would suggest that such a reaction is one whereby the trypsin directly combines with some cell constituent and inactivates it. It is also to be observed that the trypsin acts readily with several hydrogen-ion concentrations. Pepsin was entirely ineffective at all hydrogen-ion concen-

¹ The Hildebrand titration method was used to test the pH. Since the solution used is very heterogeneous in nature, no attempt was made to correct for titration error. It is quite certain any electrometric method introduces considerable error under such conditions.

² The pH was determined here by the quinhydrone method.

trations which could be used (pH 4.2—pH 8.1). Erepsin ranged from quite indifferent to slightly effective. Amylopsin, diastase, urease, and papaine did not inactivate at all. Under the same experimental conditions as were used for trypsin pangestin acted about as trypsin. Northrup's crystalline trypsin was very effective as an inhibitor of the evolution of oxygen.



Figures 5, 6, 7, 8, and 9 show the effect of trypsin on the power of the cell contents to evolve oxygen under the influence of varying hydrogen-ion concentrations. The temperature was 30° C.

DISCUSSION

It is proposed here to introduce miscellaneous statements and raise questions, rather than to cite specifically published results which support the statements or answer the questions.

Many of the proposed theories for explaining the mechanism of photosynthesis assume the formation of a peroxide of some type and the subsequent action of catalase on this to produce the free oxygen evolved. In studying the effect of temperature on the evolution of oxygen it is well to compare the effect of temperature on the destruction of the catalase activity of the same cell contents. It has been found that the temperature inactivation curve for catalase is similar in nature to that found for the evolution of O₂ by the cell contents in light, but that the temperature range is considerably higher for catalase. The critical temperature for the evolution of oxygen in aqueous solution is about 55° C. regardless of the pH, and that for catalase about 70° C.

When macerated leaves are dried, the power to evolve oxygen is soon lost, while the catalase

activity is much more gradually lost. With pH 4.5 the cell contents show a greatly reduced evolution of oxygen, and the catalase activity is markedly diminished. At pH 8.0 the cell contents soon become incapable of evolving oxygen, while catalase activity is strong. If the macerated cell contents are shaken with glass beads for thirty minutes at room temperature, the power to evolve oxygen is lost, while the catalase activity is lowered to about one tenth of its normal. Hydroxylamine strongly inhibits both the evolution of oxygen and catalase activity. Saturated NaCl and CaCl_2 inhibit both reactions but act more effectively on the evolution of oxygen.

Taking all these facts into consideration, it seems reasonable to support the assumption that, whenever green plants are capable of evolving oxygen or carrying on the process of photosynthesis, there is generally present within the cells active catalase which would be available for acting on such a substance as hydrogen peroxide if it were released.⁸ This might be interpreted as lending indirect evidence to the theory that the release of free oxygen from the plant may well be the action of catalase on a peroxide. However plausible this interpretation may seem, there is need for more confirmatory evidence. The search for a light sensitive compound, which releases a peroxide and is or is not subject to disintegration by catalase, would be a valuable contribution.

The action of enzymes upon the leaf meal, like so many questions in this field at the present time, is not readily interpreted. The fact that trypsin inactivates the meal so quickly at all hydrogen-ion concentrations indicates that this action is more likely to be a coagulating effect or adsorption of some cell constituent, rather than a true proteolysis. This does not, however, preclude the probable view that the trypsin really inactivates some constituent either attached to the chlorophyll molecule or to a substance upon which the chlorophyll itself is adsorbed. This substance could well be a native protein or a nitrogenous substance with a lower molecular weight. Since the discovery that catalase itself is a porphyrin-iron complex, it is quite possible that it might not only decompose any peroxide formed, but also play some other part in the carbon dioxide and oxygen balance between respiration and photosynthesis.

There seems to be little evidence that ground cell-free contents of the green plant absorbed carbon dioxide in light or that oxygen was absorbed in the dark. Molisch favored the view that carbon dioxide was absorbed and oxygen re-

leased during illumination by dead cells or cell contents in the absence of cells. From the studies so far made, the more probable view is that, when an active leaf is ground with sand and the cells filtered out or killed by drying, a certain amount of oxygen is bound to some substance strongly enough so that luminous bacteria cannot make use of it until a small amount of radiation of the proper wavelength is supplied. It is also to be noted that fresh leaves may be placed under a vacuum pump for a considerable time and still have the power to evolve oxygen when illuminated. Dry leaves act in the same way. That there is a definite amount of stored substance which can produce oxygen is clearly shown by the fact that once this supply has been exhausted, there is no recovery. Furthermore, when the leaves have been macerated and the cells filtered out, the cell contents give the maximum evolution of oxygen immediately; and, as time goes on, the amount of oxygen evolved decreases, depending upon the temperature, pH, and other external environmental factors. The formation of this light sensitive substance which contributes to the production of free oxygen seems to be associated with the living cell.

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DISCUSSION

Dr. Appleman: What method was used to filter out "all the cells" without filtering out the chloroplasts?

Dr. Inman: The cells were removed from this triturate by filtering through several layers of fine cloth. The filtrate was examined microscopically. This procedure did not remove many of the chloroplasts or pieces of chloroplasts.

Dr. Appleman: You claim that the inactivating action of trypsin is more likely to be a coagulating effect or adsorption of some cell constituent rather than a true proteolysis. This conclusion is based upon the effect of the hydrogen ion concentration. It would seem that the range of the hydrogen ion concentration used for both

⁸ An exception in the case of *Phormidium laminosum* was reported by Harvey (19), since it regularly grows in hot springs at temperatures at 65-73° C. and tests for catalase were negative.

trypsin and pepsin would favor the proteolysis idea rather than being opposed to it.

Dr. Inman: I fully agree that the action of trypsin may very well be a proteolytic effect and my first inclination was to give this as the only probable interpretation. After finding the coagulating effect of trypsin on the triturate it was difficult to avoid the recognition of an alternative explanation.

Dr. Appleman: The inactivation of catalase in the macerated tissue was probably due to the developing acidity, as this inactivation in many cases can be prevented or greatly retarded by grinding the plant tissue with calcium carbonate. It might be of some interest to test the effect of this treatment on the evolution of oxygen.

Dr. Inman: In tests for catalase activity the leaves were ground in buffer solutions so that the pH was controlled, and in addition pH determinations were made with the quinhydrone electrode.

Dr. van Niel: I do not quite see that the effect of trypsin would be an indication or new evidence that the chlorophyll is combined with a protein. We know that the ultimate result of photosynthesis is the result of at least two different processes, in one of which the chlorophyll takes part. Is it not possible that it is the (Blackman) enzyme factor which is destroyed by trypsin instead of a chlorophyll-protein complex, in which case the experiments do not give any evidence of the existence of such a protein-chlorophyll complex?

Dr. Inman: Since I tried to separate the proteins and other cell constituents from the chlorophyll and, like Osborne and Warkentin who tried this with spinach leaves, found it most difficult, I felt it was most reasonable to make my proposed assumption, although the explanation you suggest was not ruled out by the experimental evidence.

Dr. van Niel: I should also like to make a few remarks about the catalase situation. You mention that catalase is not active at 55° C. and that catalase is inactivated at pH 5., whereas the inactivation experiments at that temperature or with trypsin under similar conditions of acidity show correlations with the rate of decrease of oxygen production. Is it allowed to carry over the results obtained with purified catalase preparations to preparations in which the catalase is present in this extremely complicated mixture? Recent evidence obtained by Yakushiji, among others, shows that the inhibiting effect of certain factors on catalase activity depends very largely on the state of purity of the catalase preparations used.

Dr. Inman: The effect of pH and temperature on catalase activity was made directly on the triturate or aqueous extract of cell contents and

not on purified catalase solutions. A temperature time inactivation curve for catalase was made and gave a similar type of curve to the temperature time inactivation curve for oxygen production with the exception that the temperatures required for catalase inactivation were of the order of 10° C. higher in all cases.

Dr. van Niel: When you mention the observation of Harvey of the occurrence of blue green algae which contain no active catalase at a temperature of 70° C., you make the statement that these algae are growing normally. I doubt whether that is correct, because under no circumstances have I found blue green algae in Yellowstone Park at a temperature of 70°, with the characteristic blue green color; they are either colorless or yellow. I wonder whether these algae are photosynthesizing at all.

Dr. Inman: It is true Harvey does not clearly state whether *Phormidium laminosum* carries on the process of photosynthesis in a normal manner and until this is determined there is doubt that the absence of catalase in this form is an exception to the rule that all chlorophyll bearing cells carrying on the process of photosynthesis contain active catalase.

Dr. Arnold: Would you make an estimate of the rate of photosynthesis in this material after preparation, compared with the rate of photosynthesis in the leaves before you crushed them?

Dr. Inman: Using a comparative quantitative method such as I have described does not permit me to state the absolute rates of oxygen evolution but it is my impression that once the cell organization has been disturbed by crushing the cells the rate falls off fairly rapidly.

I should be inclined to reserve any definite statement as to how much the rates vary until we have obtained better data by the use of some other technique such as a Geiger counter.

Dr. Emerson: Undoubtedly it would be of great importance if you could establish the fact that oxygen production from carbon dioxide takes place in chloroplasts removed from living cells. I doubt, however, that the experiments you have reported establish this point beyond question. The method of oxygen determination is so sensitive that if a few unbroken cells had passed into your preparation, their normal photosynthesis alone might be responsible for the positive results obtained.

Dr. Inman: That the triturate contained no normally organized cells is quite conclusively demonstrated—first, by microscopic examination—second, by the fact that *Spirogyra* cells which are relatively large were broken and chloroplasts only used—third, by the fact that trypsin is not an inactivating agent when intact cells are used. One can add large quantities of trypsin to pieces

of leaf tissue, or *Chlorella* cells, and there is no inactivating effect. Thus any failure to observe cells passing through the filter would show up in the inactivation curves by trypsin.

Mr. Lineweaver: A definite answer would be given by comparing inactivation curves obtained for an extract to which a known number of cells had been added with the inactivation curves for the plain extract. If the source of the oxygen is the same in the two cases, the curves should be identical with at most a concentration effect. Have any experiments of this nature been performed?

Dr. Inman: I think such an experiment would be worth while.*

Dr. van Niel: You made the statement that you find oxygen production but no absorption of carbon dioxide and that this points to the presence of some reserve product from which oxygen would be produced. Is it not true that the test for oxygen is so infinitely more sensitive than the test for carbon dioxide that it is impossible to find out whether or not this oxygen production takes place with carbon dioxide or from reserve material?

Dr. Inman: It is true that the test for O_2 is more sensitive than the phenolsulphonephthalein test for CO_2 but passing CO_2 free air through the triturate and then into phenolsulphonephthalein for one hour gave no indication of CO_2 absorption. The triturate was illuminated and the

amount used was ten times that used in the normal tests. I am willing to admit that van Niel's question has not been entirely eliminated, however, and further experiments are necessary before a definite statement can be made that no CO_2 is absorbed during the evolution of oxygen.

Dr. Harris: To what do you attribute the observed inactivation by glass beads or charcoal?

Dr. Inman: Surface or adsorption effects.

Dr. Harris: This might well lead you to believe further that the evolution of oxygen in your studies is not due to intact cells, but is rather, as you suggest, enzymatic. If the observed evolution of oxygen is produced by intact cells, which have slipped through the cloth filter, one would not expect inactivation when the triturate is shaken with glass beads; for it seems hardly likely that intact cells would be adsorbed on the beads. On the other hand, chloroplasts, or pieces of them, might well be adsorbed on the beads, resulting in inactivation.

* Following the discussion as to the possible presence of cells in the triturate used, further experiments showed that occasional clumps of chloroplasts and a few cells sometimes passed the filters. That these cells probably had their walls injured was shown by the fact that the addition of uninjured cells to the triturate caused trypsin to fail to inactivate the solutions. Further experiments have also shown that cell-free triturate evolves oxygen. The solution was rendered cell-free by filtering and centrifuging.

THE ABSORPTION OF RADIATION BY LEAVES AND ALGAE¹

HAROLD MESTRE

Progress in the quantitative study of photochemical phenomena in living organisms has long been hampered by the difficulty of making accurate determinations of the energy absorbed during irradiation. While this problem is a basic one for all photobiologists, it is only natural that it has received most attention from plant physiologists interested in the photochemistry of carbon assimilation and in the effect of light as an environmental factor. Although the following paper is mainly devoted to a discussion of the absorption of radiation by leaves, with a briefer consideration of the problems presented by algal thalli and by suspensions of unicellular forms, it will be evident that much of it is equally pertinent to absorption by animal tissues and suspensions of animal cells.

Owing to the limitations of time and space, no attempt has been made to review the subject systematically or to give a complete bibliography. For the older literature, reference is made to Reinke (1883) and to Ursprung (1903). More recent reviews and bibliographies will be found in Stiles (1925), Spoehr (1926), and, from a more photometric viewpoint, in the experimental papers of Seybold (1932 to 1934) and of Schanderl and Kaempfert (1933). For a general consideration of photometric methods applicable to plant material reference is made to the excellent monograph of Neurnbergk (1932) and to reviews by Shirley (1931, 1935). For recent reviews of the problems and methods of photographic photometry see Harrison (1929, 1934).

While the distinction has not always been made clear in the literature, it is evident that actually there are, in general, two photometrically different problems connected with the absorption of radiation by the leaf. The first of these is the determination of the fraction of the incident flux absorbed by the leaf as a whole, which is of basic importance in establishing the general energy balance sheet of the leaf, and the second is the estimation of the absorption by specific substances within the tissues, particularly by the pigments active in photosynthesis. The first problem is distinctly the simpler of the two, and both theoretically and technically is in a much more satisfactory state than is the second. As practically everything that can be said in connection with the

first problem has a direct bearing on the second, we will first consider absorption by the leaf as a whole.

I. General Analysis of the Distribution of an Incident Light Flux

The literature reveals the interesting fact that it was not any lack of sufficient analysis of the general distribution of light incident on a leaf which for so long postponed the actual determination of an approximately correct value for the total absorption factor of a leaf, but, apparently, an inability to make the requisite measurements. As early as 1862, Simmler had clearly stated that a portion of a light flux incident on a leaf is reflected at the surface of incidence while the remainder, penetrating into the tissues and being there scattered in all directions, is in part absorbed and in part emerges through either surface. This distribution was considered in much greater detail by Reinke (1883).

To express Simmler's analysis in mathematical form, we may denote the total incident flux by P_0 , the flux reflected at the surface by P'_r , the scattered flux emerging through the surface of incidence by P'_s , that transmitted by P , and that absorbed by P_a . We may then write:

$$P_0 = P'_r + P'_s + P + P_a \quad (1)$$

as representing the general distribution of the incident flux. Dividing through by P_0 and transposing, we could then write:

$$A = 1 - (R_1 + R_2 + T) \quad (2)$$

where A , R_1 , R_2 , and T are the total absorption, surface reflection, inner reflection, and transmission factors respectively². Lumping together the two reflected fluxes P'_r and P'_s as P' , we have $P'/P_0 = R$ for the general reflection factor and

$$A = 1 - (R + T) \quad (3)$$

as the simplest possible expression for the total absorption.

It will be clear from this that the only unequivocal method for the determination of A is the

1. Contribution from the Department of Public Health, Yale Medical School, and from the Hopkins Marine Station of Stanford University. The theory here expressed is in part based on experimental work carried on at the latter institution and now being prepared for publication. Such personal experimental observations as are here mentioned are from that source.

2. The term "total absorption factor" has been used for A rather than the frequently employed "coefficient of absorption". This is to avoid confusion with the "absorption coefficient" which, strictly speaking, is represented by " K " in the expression: $P'_s + P = (P_0 - P'_r) \times 10^{-K}$. In this paper a rigorous distinction will be made between "absorption" and "extinction", the "extinction coefficient" being " E " in $P - P_0 \times 10^{-E}$, so that E is the logarithm of the opacity (P_0/P), or of the reciprocal of T .

measurement of the total incident, reflected, and transmitted fluxes in some strictly comparable manner. While it is probable that these conditions can never be fully met, it is, nevertheless, quite possible to achieve various satisfactory approximations. The instrumental problems involved in the determination of A will perhaps be clearer if we discuss them in connection with a somewhat more extended analysis of the various fluxes than is contained in equation 1.

The Incident Flux, P_0

The incident flux may be either collimated or diffuse. Under natural conditions the flux incident on a leaf is usually either actually diffuse, being light from the blue sky, from clouds, or transmitted through other leaves, or is more or less its optical equivalent. That is to say, even when the leaf is illuminated by direct sunlight its orientation with respect to the incident flux is largely random, except in the case of certain highly phototropic plants in still air. As under laboratory conditions it is frequently desirable, for optical reasons, to use a collimated flux incident normally on the leaf, it will, therefore, be necessary to consider the distribution of both a diffuse and a collimated incident flux. For measurements having to do with the general energy balance of the leaf, and in ecological problems, P_0 will usually extend over a wide range of wavelengths, being either sunlight, skylight, the total emission from some incandescent artificial source, or rather broad filtered bands. For many other investigations, especially those of photosynthesis or of photic responses, it will be necessary to work with either strictly monochromatic radiation or with very narrow spectral regions.

For the determination of the total energy absorbed from an incident flux having a wide wavelength distribution, it is evident that we must use a non-selective photosensitive element such as a thermopile. For homochromatic determinations of A , at wavelengths lying within their sensitivity range, various selectively sensitive receivers are of great utility. Owing to the possibility of enormously amplifying their output, photoelectric cells may be used with highly monochromatic light of intensities so low as to make observations with a thermopile extremely difficult and tedious. Because of their large current output, photogalvanic (*Sperrschicht*) cells are also useful under similar conditions, especially for portable instruments. On account of their liability to fatigue, and their high temperature coefficient, they are not, however, usually capable of giving as accurate results as a vacuum type photoelectric cell with a vacuum tube amplifier circuit, particularly when the latter is only used as a null indicator, or any type of cell used in a completely null instru-

ment such as Hardy's (1929, 1934) recording color analyser³. Within its range of sensitivity, recently greatly extended, the photographic plate may also occasionally be used to good advantage. It cannot, however, be too strongly emphasized that no quantitative conclusions in regard to T or R , which themselves vary as a function of wavelength, can be drawn from observations made with receivers which also, and independently, vary in their sensitivity with wavelength, unless either P_0 is monochromatic, or very nearly so, or P_0 , P , and P' are measured spectrophotometrically.

The Flux P' , Reflected from the Surface of Incidence

Both qualitatively and quantitatively the flux reflected from the surface of incidence will evidently depend not only on the characteristics of this surface, but also on the angular distribution of P_0 . Leaves range all the way from those with an extremely glossy cuticle, in which case reflection is mainly specular and tends to obey Fresnel's law⁴, to those with very finely tomentose surfaces. In the latter the reflection is almost entirely diffuse and approximately follows Lambert's cosine law. In most leaves both types of reflection will be in evidence to a considerable extent, the lower surface commonly showing more diffuse reflection than the upper one. It should be noted that in nature the lower surface may often become the surface of incidence, although, of course, less frequently than the upper one. As a rough average figure for R for the upper surface of common leaves is 0.1 (Seybold 1932b), while Shull (1929) has reported $R = 0.50$ for the lower surface of *Populus alba*, it can be seen that

³ Since the completion of this paper the full description of Hardy's modified instrument has appeared in *J. Opt. Soc. Am.*, 25: 305.

⁴ Fresnel's general equation for the intensity of the light regularly (specularly) reflected from an optically perfect surface is:

$$I_r = \frac{I_0}{2} \left[\frac{\sin^2(i - r)}{\sin^2(i + r)} + \frac{\tan^2(i - r)}{\tan^2(i + r)} \right]$$

where i and r are the angles of incidence and refraction respectively, and I_0 and I_r the incident and reflected intensities. For the condition of incidence ($i = 0$), this reduces to the special form:

$$I_r = I_0 \left[\frac{n_2 - n_1}{n_2 + n_1} \right]^2$$

where n_2 and n_1 are respectively the indices of refraction of the more and the less refractive media. It will readily be seen that as n_2 approaches n_1 , I_r will rapidly approach zero.

this may make the determination of A with incidence through the lower surface of importance.

The optical properties of the leaf surface may also be of great effect in determining the magnitude of A in other ways than by reducing the flux ($P_0 - P_r$) which actually enters the tissues of the leaf. As will be seen below, the partition of this entering flux into P'_s and P depends in part on its angular distribution and, in addition, both P'_s and P will have to make their exit from the leaf through surface tissues. To the extent which the surface of incidence is a diffusing one, it will transform a collimated incident flux into a diffuse entering flux. Within an already fully diffuse P_0 , a diffusing surface will naturally not produce any marked qualitative change in the entering flux. Similarly, a smooth surface will produce no qualitative change in a collimated incident flux, but, from Snell's law⁵, it can be seen that, if we take the index of refraction for the subcuticular tissues as only that of water (1.33), the maximum angle of incidence of ($P_0 - P_r$) on the tissues will be about 49° (omitting diffuse scattering from consideration).

The Effect of Pigment Distribution on P and P'_s

The other important portion of P' is the extremely complex flux P'_s rejected by the tissues of the leaf. This flux differs qualitatively from the transmitted scattered flux P only in the extent to which it has undergone scattering and absorption, and they will be discussed together. The intimate relation of scattering and absorption in the leaf was early recognized. Sachs (1861) commented on the "amazing" fact that although most of the pigments of the ordinary leaf are contained in discrete chromatophores, which are not arranged in a continuous layer, the pigments appeared to form just as effective an absorbing screen as though they were in solution in the cell sap. This efficiency, Sachs recognized, was due to the fact that the tissues scattered the incident light to such an extent that little of it could get through the leaf without passing through the chromatophores.

The recognition that the pigments should, in the absence of other factors, be less effective in absorbing light when aggregated into chromato-

phores than when more highly dispersed is of much theoretical importance, and the concept can be extended to include all effects attributable to the spatial arrangement of the chromatophore pigments. These will be referred to hereafter as "pattern" effects, and the "pattern factor", " p ", used as an index of the extent to which, in the absence of scattering, the absorbing power of the chromatophore pigments would, by their spatial distribution, be reduced below that of their hypothetical maximum absorbing power. The pattern factor may be defined as the fraction of the distance between the two surfaces of the leaf which would represent the mean light path through chromatophores in the absence of scattering. That pattern effects may be of considerable importance in the measurement of P'_s and P is indicated by the well known fact that not only do the chromatophores frequently change the pattern of their arrangement in the cells during irradiation, but also their form as a result of the accumulation of the products of photosynthesis. Furthermore, these effects may be a function not only of the intensity and duration of the irradiation, but also of wavelength. For a recent discussion of these reactions, reference is made to Voerke (1934), where extensive citations of the older literature will be found, and to Schanderl and Kaempfert (1934), who have actually observed the changes in T as a function of the duration of irradiation.

The "Detour" Factor

For the transmitted flux, the effectiveness of scattering in overcoming the reduction in absorption due to pattern effect is quite evidently due to the fact that it greatly increases the mean length of the light path through the tissues over that of the direct path from surface to surface, likewise increasing the mean path through chromatophores. The extent to which the light path is increased by scattering is represented by the "detour" factor " ω ". In comparing the transmission of a green and an albino leaf, Willstätter and Stoll (1918) clearly perceived the somewhat paradoxical fact that while on the one hand, the effect of scattering was to greatly increase the light absorbing effectiveness of a given amount of pigment, the presence of pigment in its turn operated to decrease the mean length of the light path. This generalization should be equally valid if we consider two wavelengths where the scattering power is substantially the same but the absorption coefficients of the chromatophore pigments are quite different. Evidently the mean light path through chromatophores will be greater in the region where the absorption coefficient is lower. As the actual absorption will be a function of the product of the absorption coefficient and the effective light path through the absorbing

⁵ Snell's law:

$$\sin i / \sin r = n_2 / n_1.$$

From this it may be seen that when i reaches its limiting value of 90° (grazing incidence), r will reach its limiting value defined by $\sin r = n_1 / n_2$. When passing from air to water, this limiting value is $\sin r = 0.7519$, or $r = 48^\circ 45'$. This is a familiar phenomenon. When passing from water to air, i and r are transposed, and all light incident at the surface at angles greater than the critical angle of $48^\circ 45'$ will be totally reflected.

substances, it is clear that, by the presence of scattering, absorption in the region of lower absorption coefficient will be increased to a greater relative extent than that in the region of higher coefficient. It would seem probable that the frequently commented on "filling up" of the extinction spectrum⁶ of the leaf in the green, as compared with the spectrum of extracts of the leaf pigments, is in part due to this effect, the balance being mainly attributable to the fact that the longer light path permits a larger proportion of the scattered flux to become directed toward the surface of incidence where the unabsorbed portion will appear as P'_s . The great increase in ω possible with low pigment concentration, would also seem to furnish a logical explanation for the extraordinary photosynthetic efficiency of the green pigments in young leaves and in yellow varieties. For example, Willstätter and Stoll have reported that in a yellow variety of *Ulmus*, with a chlorophyll concentration of only 1/13.5 of the normal green form, the assimilation of CO_2 , in grams per hour, was 0.098 while that in the normal green form was only 0.111.

The Partition of the Entering Flux Into P and P',

Evidently the partition of $(P_0 - P'_r)$, the flux actually entering the tissues of the leaf, into what may be called "potential" P'_s and P , will depend primarily on the scattering power of these tissues. Denoting this by σ , it is clear that "potential" P'_s will increase rapidly with σ , while "potential" P will decrease. The final magnitudes of the emergent fluxes will depend upon this partition, which will also be a function of the angular distribution of $(P_0 - P'_r)$, and on the extent to which the two "potential" fluxes have been attenuated by absorption. As absorption also increases with σ , for both fluxes, it can be seen that while P'_s changes as the difference of two functions of σ , P will vary as the sum of two similar functions. It should further be noted that both partition and effective absorption are a function of the spatial distribution of scattering power and of absorbing power in the tissues, that is to say, of the histological characteristics of the leaf.

With a collimated incident flux, it is obvious that only light which is deviated more than 90° from its original direction, whether at the surface

or in the tissues, can possibly appear as P' . On the other hand, with a diffuse entering flux, a comparatively small deviation will cause part of the entering flux to emerge through the surface of incidence. This effect will be of greater relative importance with high absorbing power than with low. On the contrary, the probability of emergence as transmitted flux is reduced. It is, therefore, to be expected that with diffuse irradiation T will be lower, and the ratio R/T will be greater than with a collimated incident flux, especially when the absorbing power is high and the surface of incidence is not highly diffusing. It is quite possible that there will be little difference in A with diffused and collimated P_0 , that is to say, the sum of R and T may remain approximately constant. Owing to the difficulty of determining the total P' for diffuse incident light, no experimental data are as yet available which are adequate for strict comparison with measurements of the total P' for collimated light. Measurements by Seybold (1933a), in which filtered radiation and a photogalvanic cell were used, indicated only a slight difference between T for collimated and for diffuse illumination.

The Nature of Scattering in the Leaf

The light scattering power of the tissues of the living leaf would appear to be due mainly to refraction and reflection, including total reflection, at interfaces, rather than to scattering by small particles. That this is the case is indicated by the very slight variation in the transmission of colorless plant tissues at different wavelengths in the visible. Shull (1929) has found values for R for the tomentose lower surface of the leaf of *Populus alba* of practically 0.50 at all wavelengths from 700 to 430 $m\mu$, the slight increase to a maximum of 0.53 in the yellow green evidently being due to a small contribution from P'_s . If any important portion of this scattering were due to particles small in relation to the wavelength of the light used, and therefore following Rayleigh's equation⁷, it should make its presence evident by a considerable increase at the shorter wavelengths due to the operation of the inverse fourth power term. The presence of such an increase in the case of "albino" leaves is undoubtedly due, as

⁶ The term "extinction spectrum", representing the variation with wavelength of the extinction coefficient, $E = \log (1/T)$, is here used instead of the term "absorption spectrum" commonly used in the past to describe the same function. While in true solutions the two would be synonymous, it is evidently necessary, in the presence of scattering, to distinguish in some way between simple diminution of the transmitted flux, and actual absorption of energy.

⁷ Rayleigh's equation may be written in the following exponential form for the transmitted flux for unit light path:

$$P/P_0 = e^{-\frac{k_2 N d^6}{\lambda^4} \left[\frac{n_2^2 - n_1^2}{n_2^2 + 2n_1^2} \right]^2}$$

where k_2 is a constant, N is the number of scattering particles per unit volume, d is the diameter of the particles, λ is the wavelength, and the other symbols have their usual significance.

pointed out by Shull, to the presence of yellow pigments. The observation, made both by Pokrowski (1925) and by Shull, that some leaves do not exhibit the expected increase in reflection factor at wavelengths longer than the red absorption maximum of the chlorophylloid pigments, may indicate either that in some leaves σ is very low in that region, or that they contain some unreported pigment absorbing there. This can, of course, be settled by determining both R and T for these leaves homochromatically.

A further indication of the major role of reflection-refraction scattering is to be found in the many observations made with leaves in which the normally air-filled tissues of the parenchyma are "injected" with water by evacuating the air while submerged. This process also results in the removal of the interstitial air in the surface of tomentose leaves. That injection greatly increases the transmission has long been known, and the practice has been widely employed by investigators of the transmission spectrum on that account. It is, of course, evident that this increase in transmission can only be due to a reduction in scattering power brought about by the substitution for air of a substance of higher refractive index. Regular reflection at interfaces follows Fresnel's law, and refraction and total reflection is governed by Snell's. From the form of these laws^a, it can be seen that the magnitude of both effects will depend very acutely on the magnitude of the difference between the indices of refraction (n_2 and n_1) of the two substances forming the interface. Except in the presence of air, and in the case of oil droplets and similar highly refractile cell inclusions, it is probable that n_2/n_1 will not greatly exceed 1.40/1.33. In tissues containing air the ratio of the indices will be nearer 1.40/1.00 if we omit from consideration the thin surface film of water which plays no part in the net effect. While n_2/n_1 is a function of wavelength it will not vary rapidly with most biological substances.

The recent thermopile measurements of Seybold (1932b) in the spectral regions of higher transmission, show that T_i , the transmission factor for the injected leaf, may easily be of the order of two and a half times greater than T . This is the case both in the normal green and in the albino leaf. Seybold's accompanying photoelectric cell measurements of R , which are not strictly comparable with those of T , as the radiation measured was not monochromatic, might appear to indicate that this increase in T in the injected leaf takes place wholly at the expense of R , as it is greater when R is high. Unfortunately, Seybold was not able to determine R , with his

apparatus. It would seem, however, that the simple relation suggested by Seybold, $R + T = R_i + T_i$, could not, on purely analytical grounds, be expected to hold except under conditions of zero absorption. In general, $(R_i + T_i)$ should be greater than $(R + T)$, and the difference be a measure of the decrease of A resulting from changes in the σ of the tissues as a function of n_2/n_1 .

The Leaf Surfaces and the Emergence of P and P'

It is evident that a diffusely reflecting surface will act as a "light trap", returning a portion of the scattered fluxes to the tissues. Less obvious is the action of specularly reflecting surfaces. Again from Snell's law, it can be seen that all light incident on such a surface at an angle greater than 49° , or probably even less, will be totally reflected and returned to the tissues. This would indicate that, for a fully diffused flux within the leaf, a very smooth and shiny surface will be at least as efficient a light trap as the mostly finely tomentose surface known. At the surface of incidence it will, in fact, be peculiarly efficient. It will prevent the emergence of all flux which has not penetrated deeply enough to have become deflected through the large angle (130° or more for a normally incident collimated flux) required to bring it to the surface at an angle of incidence less than 49° . This, and the lack of diffuse surface reflection of white light, accounts for the extremely dark green color of the upper surface of most glossy leaves. The importance of this effect in connection with the angular distribution of P and P' is evident.

The Angular Distribution of P and P'

As will be seen below, a knowledge of the angular distribution of P and P' may be of much importance in the measurement of these fluxes. From the preceding analysis it is clear that the angular distribution of both fluxes will depend on the angular distribution of the incident radiation, on the nature of the leaf surfaces, and on the scattering power and thickness of their tissues.

The more diffusely reflecting the leaf surfaces, and the higher the σ of the tissues, the more nearly will both fluxes approach conformity to Lambert's cosine law. The opposite extreme will be reached with a very glossy leaf irradiated normally with a collimated flux. Here the angular distribution of intensity will depart widely from Lambert's law. This will be particularly so in the case of P' , as not only will there be a large component of P' , normal to the surface, but the operation of the upper surface on P' , will be

^a. See footnotes 4, p. 192, and 5, p. 193.

more marked than that of the usually less glossy lower one on P.

Both of the above conditions are evident in the data of Seybold (1933), to whom we are indebted for the only existing determinations of the angular distribution of these fluxes. The greater amount of flux emergent at small angles to the normal in shiny leaves is very clearly seen by comparing the form of Seybold's distribution curve for P' of the glossy *Prunus laurocerasus* with that of *Tropaeolum majus*.

Other Components of P and P'

Two other groups of fluxes should be mentioned here as they also may be components of the measured fluxes P and P' . These are the fluorescence fluxes P_f and P'_f , and the infrared emission P_e and P'_e , the primes, as usual, indicating emergence through the surface of incidence. Although its existence was long disputed, red fluorescence from the chlorophylloid pigments can be observed in most leaves with the aid of a spectroscope, or suitable filters, to exclude the far brighter scattered and reflected fluxes. Except in extremely thin forms and unicellular algae, only P'_f is sufficiently bright to be observed even under the most favorable conditions. In spite of the important bearing of this chlorophylloid fluorescence on the problem of photosynthesis, the total flux is so small that it has not yet been possible to determine the fluorescence factor ($F = (P_f + P'_f)/P_e$) attributable to these pigments. It should be noted that in addition to the green pigments other substances (phycoerythrin, phycocyanin, alkaloids, etc.,) may be present and with suitable excitation, will fluoresce.

The infrared emission has been little studied as radiation, being usually lumped with conduction and convection losses from the leaf. In the determination of A with thermopiles, or other non-selective receivers, most of the effort, heretofore, has been devoted to avoiding the measurement of these as a part of P and P' . *A priori*, it would appear to be probable that P_e and P'_e were spectrally quite complex, consisting of vibrational quanta, radiated from photically excited molecules, as well as of much longer wavelength thermal radiation. This does not appear to have, as yet, been investigated experimentally although it could easily be done. It would seem possible that the radiation of vibrational quanta might be an important part of the energy distribution mechanism of the leaf.

Physiological and Pathological Factors

The effect on the pattern factor of changes in the position or shape of the plastids during irradiation has already been mentioned. Similarly,

changes in the amount and distribution of intercellular gases, or in the degree of hydration of cell colloids, may be expected to cause a variation in σ . During growth there are marked alterations in σ due to changing structure and to hydration, as well as to variations in the light absorbing power with changing concentrations of the various pigments. Among the changes in structure with age may be mentioned that of hairiness. The data of Linsbauer (1901) indicate that, in the spectral range to which photographic paper is sensitive, a very young quince leaf had its transmission cut in half by the surface hairs which are abundant at that stage of development, yet only a little later they were almost without effect. Coblenz (1912), and Pokrowski, and Shull all found that R was progressively lower with increasing age.

In addition to these and other changes as a function of age, irradiation, or water relation, it is evident that the most marked variation can exist between leaves of the same species of plant developing under different environmental conditions or because of genetic differences. Pathological changes, such as the presence of mildew, may cause marked increases in R , as Shull has shown. Intense irradiation in the shorter wavelengths of the visible, or with lower intensities of ultraviolet light, may bring about rapid irreversible changes in the absorption coefficients of the pigments. Excessive heating while under observation may do the same and may affect both p and σ as well.

II. *The Determination of A*

Even with the most exact specification of the biological material, and the most careful treatment during measurement, there must always be a considerable doubt as to the extent to which the material of one investigator is comparable with that of another. If to this we add the employment of a wide variety of photometric techniques, often faulty from a physical standpoint, and the use of incident radiation of a wide range of wavelengths and differing degrees of spectral purity, it frequently becomes impossible to say whether a disagreement in the data of two investigators is due to the material or to the method. This situation, so common in biology, inevitably leads to endless and frequently unprofitable controversies. These can best be avoided through a thorough clarification of the photometric problems involved, which should lead to the development and use of adequate and comparable instrumental techniques.

The Photometric Problem

From the preceding analysis it would appear that the photometric problem may be stated as

follows. For the determination of A , under any given set of conditions, either the three fluxes P_0 , P , and P' must be measured separately, or the ratios P/P_0 and P'/P_0 , giving T and R . Let us denote the absorption factor for a normally incident P_0 by A' , and the corresponding factor for a diffuse P_0 by A'' . For A' then, we need to measure a collimated P_0 , for A'' an evenly diffuse P_0 , and, for both, the diffuse fluxes P and P' , which vary in angular distribution with different material and with the angular distribution of P_0 , or else the above mentioned ratios of these fluxes.

As already mentioned, the only unequivocal method of determining either A' or A'' is the measurement of the total incident, transmitted and reflected fluxes. Unfortunately this can only be approximated and in these necessary approximations lie most of the difficulties of the problem. As a detailed general discussion of photometric methods is not possible within the scope of this paper, the following remarks will be confined to rather specific considerations and to pointing out certain more or less obvious pitfalls.

Historically the main difficulties appear to have first centered around the measurement of diffuse fluxes, and then about the spectral selectivity of the material, or of the photosensitive receivers employed. The second has already been sufficiently commented upon, but the first warrants a brief discussion. The general statement may be made that in photometry we never actually measure any given flux in its entirety, but only some fraction of it, which we will designate by " q ". This fraction, " q ", may represent the net effect of many different factors, principally losses by reflection or absorption in optical systems, lack of sufficient angular aperture in the photometer, and the efficiency of the photosensitive element. In homochromatic photometry, or in total energy determinations with non-selective receivers, this latter may be considered constant. The first two factors may be made constant, or more or less adequately corrected for, but problems arising from the angular distribution of the fluxes to be measured still remain.

The Determination of R/T and of A with the Spectrophotometer

The isolated pioneer attempt of Vierordt (1873) well illustrates the magnitude of the error which can arise through neglecting the effect of angular aperture. Using a visual spectrophotometer and direct sunlight as the incident flux, Vierordt tried to determine T for normal incidence and observation, and R for 45° incidence and observation. Let us denote the fluxes actually traversing the instrument, and so compared, by qP_0 , $q'P$, and $q''P'$. Owing to the relatively small angular aperture of the instrument, and to

the fact that P_0 was practically collimated, while P and P' were diffuse, it is evident that q must have been very much larger than q' and q'' . For this reason the values found for T and R were absurdly low: for example, only about 0.0005 for T and 0.0008 for R for a maple leaf (*Acer platanoides*) in the yellow green between the D and E lines. These values are about 1/100 of what they should be, and would represent the leaf as being very nearly black.

It is unfortunate that the manifest incorrectness of these determinations for T and R , which make them useless for the estimation of A , wholly obscured the important fact that significant values for the ratio R/T could have been computed from them. This is, of course, due to the fact that q' and q'' are of the same order of magnitude and that q is eliminated in the division. As a matter of fact, the values for R/T derived from Vierordt's data are probably of much the same order of accuracy as, and are in good general agreement with, those calculated from the recent data of Pokrowski (1925) and Seybold (1932b).

It is interesting to note that the method used by the Russian physicist Pokrowski only differed from that of Vierordt in the use of an MgO surface to obtain a diffuse P_0 for the determination of R , so making q and q'' more nearly of the same magnitude, and of an opal glass plate to perform the same function for q and q' in the determination of T . In this way Pokrowski obtained realistic values for T and R , but it must be noted that the troublesome matter of angular distribution was not wholly avoided by his methods. In the determination of R , P_0 was incident normally on both the sample and the standard, and qP_0 was compared with $q''P'$ at an angle, θ , of 10° from the normal. As the MgO surface is almost perfectly diffusing, the angular distribution of the diffused P_0 will closely follow Lambert's cosine law. We have seen, however, that P' may depart widely from this condition and that, in general, for small values of θ , we can expect q'' to be greater than q , giving rise to values of R which are to that extent too high. While, for a very glossy leaf, the specular component of P' , would in large part fail to enter the spectrophotometer slit at $\theta = 10^\circ$, we can, nevertheless, still expect q'' to be greater than q owing to the effect of total reflection at the surface on the angular distribution of P'_0 .

For the determination of T , Pokrowski placed an opal glass in front of the slit of his spectrophotometer, thereby insuring that both $q'P$ and qP_0 should be evenly diffused. While this eliminates any effect of the instrument on q and q' , it still does not entirely avoid the effect of differences in angular distribution, as the opal glass is,

in one case, illuminated by the collimated flux P_0 , and, in the other, by the diffuse flux P . As the transmission factor for opal glass will be higher for a collimated flux (cf. Dreosti, 1931), q will be greater than q' , and the values obtained for T will be lower than their correct value. It should be noted that the errors in determining T and R are in opposite directions, which would tend to give a correct value for A' . To Pokrowski should be given the credit for the first explicit statement of equation 1, long, however, implicit in the biological literature.

The Use of Spherical and Ellipsoidal Mirrors

The first comparable determinations of R and T ever reported were apparently those of Coblentz (1912) made in the course of his extensive study of reflection. They, however, did not find their way into the biological literature until 1929, when Shull performed this service. While Coblentz determined R for the leaves of some nine species, for a collimated flux incident nearly normally, he only reported values for T for two of these, the lilac and the black locust. For the lilac he found $R = 0.236$, and for the black locust $R = 0.239$. His corresponding values for transmission are $T = 0.196$ and $T = 0.207$ respectively, which are stated to be "corrected for reflection". If, as seems indicated, these values are calculated for $P/(P_0 - P')$, the T values corresponding to P/P_0 would be only 0.150 and 0.158. Using Coblentz's figures for T , we would have, for the lilac, $A' = 0.568$, and for the black locust, $A' = 0.554$. With the above calculated values for T , these would become 0.614 and 0.603 respectively.

References to Coblentz's paper indicates that these determinations and most of the others, were not made with monochromatic light at 600 $m\mu$ as might be inferred from the table cited by Shull, but with the radiation from an acetylene flame filtered through 2.5 cm. of 1 percent cupric chloride. This cuts off at 700 $m\mu$ and gives an energy curve in the visible similar in shape to the solar energy curve, with a maximum around 600 $m\mu$. Three determinations of R with fairly monochromatic light centering around 540 $m\mu$, gave values only from 0.007 to 0.026 above those for the total visible, which would indicate that most of the P' in either case was in the green.

An additional measurement on a tulip leaf with filtered radiation, including the visible and the infrared to 1.4 μ , with a maximum energy at 950 $m\mu$, showed an R of 0.380 as compared with 0.219 for the visible alone. As the long wave cut-off was effected by 2 cm. of water, it is evident that the increased R was due to low absorption in the region lying between the chlorophylloid red absorption maximum and the water bands. A single measurement with radiation from a Bun-

sen flame, with 90 percent of the energy at 4.4 μ , showed an R of only 0.056. This is presumably due to intense absorption by the water of the leaf tissues, the absorption coefficient of water being very high in this region.

It would appear from Coblentz's paper that all of the measurements used in determining the above values for R were made with a technique quite different from that indicated by Shull, which related only to some earlier measurements. As this method is of interest, and has not been described in the biological literature, it will be briefly outlined. The foundation of the apparatus was a surface silvered hemispherical glass mirror 10 cm. in diameter. Through a small polar aperture in the mirror, the image of an illuminated slit was projected almost normally on the specimen which was held in position in the equatorial plane. The image was formed at a distance of 3 mm. from the center of curvature of the mirror, so that all light reflected from the specimen was brought to a focus on a surface thermopile in the conjugate position on the opposite side of the center.

The instrument was calibrated for losses of P' through the aperture for the incident flux, and for loss by absorption by the mirror. It should be mentioned that owing to slight changes in the absorption coefficient of the silver with wavelength, a small error may have entered here, when using extended spectral regions and such highly selective material as leaves. It is probable that the recently developed aluminum surfacing would be preferable to silver. By placing the thermopile in the position later occupied by the specimen, qP_0 was measured directly. Coblentz assumed that the difference between the loss of energy by reflection at the glass, or rock-salt, cover of the thermopile when measuring qP_0 , which was practically normally incident and collimated, and the loss when measuring $q'P'$, which was diffusely incident, could be neglected. With this assumption it is difficult to agree, as from an application of Fresnel's law it can be seen that the reflection loss should be considerably greater with the diffuse flux. The fact that the flux was not incident normally on the thermopile is also to be considered. It is perhaps possible that the omission of this correction factor may account for the values of 0.863, reported for the R of MgO with filtered visible radiation, and of 0.852 for $MgCO_3$. These are about 10 percent low compared to the usually reported values ranging from 0.95 to 0.98 (cf. Taylor 1934). If this difference was due to the above suggested cause, and not to the physical state of the preparations, it would seem that Coblentz's values for R should be correspondingly increased.

Coblentz's values for T are not strictly com-

parable with those for R , the measurements having been made by placing the specimen in front of the thermopile with a small air gap to avoid thermal conduction from the leaf to the thermopile cover. No correction appears to have been made for the increased reflection loss due to the difference between the angular distribution of P and P_0 , and, it should be remarked, no such correction has been applied by any one of the investigators, from Brown and Escombe (1905) to Schanderl and Kaempfert (1933), who have employed receivers covered with glass plates for the measurement of $q'P$. In all cases it would appear that there should be a revision upward of the reported values for T (except where other factors, such as thermal conduction, would indicate a greater revision downward). There would appear to be no reason why Coblenz's apparatus could not be arranged quite simply to permit the determination of T in a manner strictly comparable to that used for R , and the use of a smoked MgO standard surface for measuring $q'P_0$ would appear to be more or less unobjectionable as, in this case, the magnitude of q , q' and q'' is much less dependent on angle than on absorption. Also, for monochromatic irradiation, various selective receivers could be employed.

Seybold (1932b) has also made similar use of a mirror to determine R . In this case the mirror was part of a prolate ellipsoid of revolution, and the specimen, backed with black paper and mounted on the surface of a photoelectric cell, placed in position at one focus and illuminated. The flux $q''P'$ is collected by the photoelectric cell on its way to the other focus of the ellipsoid. As already mentioned, the actual observations are limited in interest, as they were made with rather wide filtered bands and a selective receiver. There is, however, no reason why the apparatus should not be used to advantage with monochromatic radiation. qP_0 is measured by substituting an MgO surface for the specimen. This would appear to be unobjectionable in this case also for the reason given above. The apparatus does not, in its present form, permit of directly comparable determinations of R and T .

The great potential advantage of mirror methods lies in the fact that all of the transmitted or reflected flux, except that lost by absorption, or by reflection at the photosensitive element, is measured. This not only largely eliminates the difficulties arising from non-uniform angular distribution, but also means that much larger fluxes are available for measurement than in instruments of smaller angular aperture.

The Use of the Ulbricht Sphere

The Ulbricht sphere offers great possibilities if used with due regard for its optical characteris-

tics, and those of the leaf. As the angular distribution of P and P' only approaches obedience to Lambert's cosine law as a limit, and, with P' in particular, it may depart very widely from this law, it is evident that the Helmholtz reciprocity law cannot be assumed to hold. That is to say, diffuse irradiation, and measurement of the brightness normal to the surface as compared to that of a MgO standard, is not equivalent to normal irradiation and measurement of the integrated diffusely reflected flux.

The only actual use of the sphere which appears to have been made was in the already mentioned work of Shull, in which R was determined for a large number of leaves. The instrument, in this case, was the Keuffel and Esser "color analyser". Here the brightness, normal to the surface, of the diffusely illuminated specimen and $MgCO_3$ standard, are compared by means of a visual spectrophotometer. With the sphere used in this way, it is evident that it becomes an instrument of no greater angular aperture than that of the spectrophotometer used, and that the remarks made in connection with Pokrowski's method will, in general, apply. The exclusion of the specularly reflected component of P' , is even more rigid in this case than with Pokrowski's arrangement. It is evident that, in this arrangement, no particular benefit is derived from the use of the sphere. In spite of the interest and value of Shull's observations, it is obvious that the values obtained for R cannot be used for the determination of either A'' or A' . Furthermore, the apparatus cannot be used for the determination of T .

The great utility of the Ulbricht sphere in the photometry of the leaf clearly lies in its power of integrating the diffuse flux radiated by the specimen, or standard, illuminated normally, or nearly so, through an aperture. In this way the effects of varied angular distribution may be wiped out, and q , q' , and q'' made substantially equal. With the sphere used in this manner measurements of P , strictly comparable with those of P' , may readily be made by placing the specimen over the illumination aperture, while the standard surface remains in place in the sphere wall.

In a previous paper (Mestre, 1930), the potential usefulness of the original form of Hardy's (1929) recording spectrophotometer for measuring leaf transmission spectra was commented upon and certain deficiencies, mainly in regard to the slit width required, were pointed out. Nuernbergk (1932) also realized the great possibilities of this instrument for determining R , if it could be modified to include an Ulbricht sphere. In the elegant present modification of Hardy's instrument⁹ all of these objections have been met, and

⁹. See footnote 3, p. 192.

it would now appear to be an almost ideal instrument for the determination of A' . A single possible change which might be required in order to avoid loss of the specular component of P' , in glossy leaves, is to incline the sample at a slight angle to the incident beam. If the Ulbricht sphere is of sufficient size in relation to the illumination aperture, and the incident beam not too strictly collimated, this would not be necessary.

One other possibility of the sphere, which arises out of the foregoing remarks concerning the specular component of P' , may be briefly mentioned. It is that with a suitable sphere this component might be determined by the difference between two successive measurements of P' , the first with exactly normal incidence, and the second with the specimen inclined just enough to prevent the exit of the specular reflection through the illumination aperture. The incident light should, of course, be collimated as strictly as possible.

Goniophotometric Methods of Determining A' and R/T

In concluding this discussion of A' , we will refer only to two methods suggested by Nuernbergk (1932), and actually applied to leaves by Seybold (1933), by which the angular distribution of P and P' is determined in a given plane at right angles to the specimen. The results of this study have already been mentioned in connection with the discussion of the angular distribution of P and P' as experimental confirmation of the theory there expressed. Valuable though these observations are in giving a picture of the general character of the angular distribution, it must be pointed out that considerable refinement will be necessary before they may be made the basis of quantitative determinations of R and T , which could be used for the estimation of A' .

In the photographic method, a narrow band of light (Seybold used filtered light, almost wholly of 540 $m\mu$) was thrown on the specimen and the P and P' allowed to fall on an encircling band of moving picture film. The developed image was then run through a Zeiss recording densitometer. With the subsequent computation of the ratio R/T , in which planimeter integrations of the areas below the two densitometer traces are taken as proportional to P' and P , it is not possible to agree. This is not only because of the omission of the corrections usual in photographic photometry, but also because of the failure to integrate over the entire solid angle (2π) through which each flux is distributed. In view of the manifest differences in angular distribution this operation cannot be omitted. Owing to the many difficulties inherent in photographic photometry, Seybold's second method would seem more promising.

In this method a photogalvanic cell was caused to travel in a circular path about the irradiated specimen, and the amplified galvanometer deflections recorded photographically. In addition to making the same criticism in regard to integration in one plane only, it should be pointed out that it is also necessary to correct for the fact that the photocell apparently was not rotated on a great circle about the specimen, but on a smaller circular path in a plane parallel to the plane of the great circle passing through the slit. It may also be suggested that the resolving power of the apparatus could be greatly increased by using a slit in front of the photocell or, preferably, by adding an optical system of high directional selectivity to the photometric unit.

III. *The Determination of the Absorption Factor of the Plastid Pigments*

The total absorption factor must be the sum of a large number of partial absorption factors, representing absorption by all of the varied constituents of the leaf tissues. If we denote the partial absorption due to the plastid pigments by A_p , and all the remainder of the absorption due to cell walls, water, cytoplasmic pigments, etc., by A_t , we may write:

$$A_p = A - A_t \quad (4)$$

Outside of the direct value of the determination of A for investigations of the energy balance of the leaf, it is evidently of basic importance as a primary step in the estimation of A_p , which is of such vital interest to plant physiologists on account of its relation to photosynthesis. This ardent desire for working values of A_p , the already discussed instrumental difficulties arising from the angular distribution of P and P' , resulting in impossibly low values for T when determined from P/P_0 measured with an ordinary spectrophotometer, and the complete inability to measure R , led to a long series of attempts to approximate A_p by some other means.

In all of the earlier attempts this involved either an explicit or an implicit exclusion of R from consideration. In most attempts there was an additional negation of one or more factors characteristic of the leaf or algal thallus. The attempt of Timiriazeff (1903) to estimate A_p on the basis of the absorption by an extract of the pigments from a given leaf area, traversed by a collimated beam of light of this same cross-sectional area, constituted not only a total ignoring of all of the optical properties of the leaf, but also the implicit assumption that the absorption of the extracted, and the *in vivo*, pigments varied in the same way as a function of wavelength. This erroneous assumption will be further discussed in connection with the observations of

Wurmser (1921), and of Warburg and Negelein (1923).

Only slightly less definite a denial of the structure of the leaf is implied in the assumption that A_p can be estimated by comparing the transmission factor for an albino leaf with that of the normal green variety, or those of the "white" and "green" portions of the variegated leaf. In its crudest form this results in the expression:

$$A_p = T_{alb} - T \quad (5)$$

Taking the data of Brown and Escombe (1905), showing $T_{alb} = 0.255$ and $T = 0.213$ for the leaf of *Negundo aceroides* (*Acer negundo*), Weigert (1911) applied this equation and obtained the absurd figure of $A_p = 0.042$. It was, nevertheless, used by him to calculate that 98 percent of the energy absorbed by the plastid pigments was used in photosynthesis.

By comparison with the data of Seybold (1932b) for this leaf, it would appear that the excessively low value for A_p obtained by Weigert was due to the fact that much of the transmission for both the albino and green, in Brown and Escombe's observations, was in the near infrared, between the red chlorophylloid absorption maximum and the water bands at 1.4μ . In any case, the objection, first raised by Willstätter and Stoll (1918), that the greatly increased mean light path in the albino leaf will result in such a great increase in absorption by its tissues that its transmission cannot be taken as a direct measure of A_t in the normal leaf, will apply here. Evidently the high value of ω will also effect quite a different partition between R and T .

Brown and Escombe, however, in their endeavor to obtain a figure for A_p which would appear more in harmony with those of Timiriazeff (0.20 to 0.29, by the extraction method), considered that the difference between the two transmissions should be expressed as a fraction of the flux transmitted by the albino leaf, which they assumed to represent that of the tissues alone. This resulted in an expression of the form:

$$A_p = (T_{alb} - T)/T_{alb} \quad (6)$$

which, with the above data, gave $A_p = 0.165$, a figure which appealed to them as being in reasonable agreement with Timiriazeff.

This expression has been used as recently as 1934 by Schanderl and Kaempfert, who obtained with it such apparently reasonable values as 0.70 for filtered "white light", and 0.63 for "green-yellow". In addition to ignoring R , and the optics of the leaf, equation 6 also involves the implicit assumption, as pointed out by Willstätter and Stoll, that all of the pigment is located on the underside of the leaf or, at least, absorbs quite independently of the leaf tissues. It is, therefore, fundamentally erroneous, and values

for A_p derived by its use can have no actual validity, however reasonable they may appear to be.

Reinke's Method

Reinke (1883, 1886), who obviously had an excellent understanding of the optics of the leaf, initiated a procedure which at first sight would appear to differ from that of Brown and Escombe. He assumed that the difference between the extinction coefficient of a normal leaf or thallus, and the extinction coefficient for the same tissue with the pigment extracted by alcohol, represented the absorption coefficient for the plastid pigments, which we will represent here by K_p ¹⁰. This, in our notation, would be written:

$$K_p = E - E_w \quad (6)$$

from which, continuing to ignore R_t and R_s :

$$A_p = 1 - 10^{-K_p} = 1 - 10^{-(E-E_w)} = (T_w - T)/T_w \quad (7)$$

This is identical in form with Brown and Escombe's second expression, which was so justifiably criticised by Willstätter and Stoll.

This manner of estimating A_p , while obviously incorrect, has been widely used and, with certain types of material, gives values which are apparently of a rational order of magnitude. It will, therefore, be worthwhile to consider it somewhat further. The most evident reason for the fairly realistic values obtained is the elimination of the usual large error arising from angular aperture. This is due to the fact that only the ratios of the measured fluxes are used, as can be seen by rewriting equation 7 as follows:

$$A_p = 1 - T/T_w = 1 - (q'P/qP_o)/(q'P_w/qP_o) = 1 - q'P/q'P_w \quad (8)$$

where the values of q' for P and P_w may be considered to be very nearly equal. The values found for E and E_w are, of course, much too high, and the corresponding values of T much too low, due to the fact that q is very much larger than q' , but, just as in the case of Vierordt, the ratios are substantially correct.

From equations 4 and 2, we may write for the correct value of the pigment absorption factor:

$$A_p = 1 - (R_t + R_s + T + A_t) \quad (9)$$

If Reinke's expression were correct, we would then be able to write:

$$R_t + R_s + T + A_t = T/T_w \quad (10)$$

which is obviously not true. The origin of the apparently reasonable values obtained for A_p by Reinke's expression is now clear. From the pre-

¹⁰ The notation here used is simply an extension of that in footnote 2, p. 191, to partial absorptions and transmissions.

vious analysis it is plain that the left hand term of equation 10 will increase with decreasing A_p , as the sum of the three factors R_s , T , and A_t , which are all increasing, R_t not being affected. The right hand term will also be increasing with T , and this increase will be multiplied by the factor $1/T_w$, which may be a relatively large number when q' is small. Wurmser (1921), who used Reinke's method, with the single exception that T_w represents the transmission factor of a sun-bleached thallus of *Ulva* instead of an alcohol extracted one, found a uniform value for E_u of practically 1.00, which would give $1/T_w$ a value of 10. It may be remarked that it is obviously impossible to multiply all of the various values of T by a constant factor and obtain correct values at different wavelengths, as R_s , T , and A_t do not vary as identical functions of the absorbing power.

The incorrectness of Reinke's expression can also readily be seen by examining the boundary value as pigment absorption increases. From equation 9 it is evident that A_p can never have a value higher than $1 - (R_t + x)$, where x represents the sum of the small residual values of R_s and A_t , which do not approach zero as a limit. On the other hand, the limit approached by T with increasing absorption is zero, so that the limiting value of A_p in Reinke's equation is unity. This is impossible.

It should also be observed that Reinke confined his observations to water injected leaves and to the flat thalli of algae, all measured while immersed, and hence under conditions where R_t is at a minimum. While this is a normal condition for marine algae, it is evident, from the previous analysis of the effect of injection on A , that A_p for an injected leaf must be less than for a normal air-filled one. The fact that in thin algae and injected leaves T is much more important than R_s or A_t also contributed to covering up the incorrectness of the expression.

The Methods of Warburg and Negelein

The work of Warburg and Negelein (1922, 1923), which still constitutes our chief source of information regarding the photochemical efficiency of the plastid pigments, well illustrates the difficulties inherent in the estimation of A_p . Strictly speaking, they made no effort to determine this factor, as they merely endeavored to set up a system wherein they could consider $P_a = P_o$. This they did by irradiating a thick suspension of *Chlorella* cells through the bottom of a manometer vessel, the other surfaces of which had been, in so far as possible, silvered on the outside.

In their 1922 experiments they made no attempt to determine P' , assuming it to be negli-

ble, and assured themselves, in two different ways, that P actually could be neglected. The first of these was that on doubling the cell concentration, they found no increase in the rate of photosynthesis. The second was that an alcoholic extract of the cell suspension, of the same volume and area, apparently showed complete absorption when placed in front of the bolometer used for the measurement of P_o .

In the work reported in 1923, where monochromatic (578, 546, 436 $m\mu$) and filtered (690-610 $m\mu$) radiation was used, an effort was made to show that P' actually was negligible. To do this they covered one half of the lower surface of the the manometer vessel, containing the cell suspension, with tinfoil coated with MgO , and illuminated both halves uniformly with a collimated beam. The brightness of the MgO surface was then reduced by interposing smoked glass filters until it matched that of the uncovered area as observed from the side. The transmission factor of the filters was then determined with the bolometer and this taken as a measure of R . This was done for each of the above spectral regions with a resulting value of 0.005 at 546 $m\mu$, and indeterminate values less than this for the other wavelengths.

On the basis of these data, Warburg and Negelein concluded that R was negligible in comparison with other sources of error. It should be noted, however, that R is probably actually very much larger than indicated by the above technique, mainly because of the fact that all P' incident on the glass bottom of the vessel at an angle of more than 49° will be totally reflected. The solid angle defined by this angle of incidence is only about 35 percent of the solid angle of 2π steradians over which P' is distributed. The approximately one third of P' which is able to emerge from the vessel will, furthermore, be rapidly decreasing in intensity as the angle of view departs from normal, and a comparison of the brightness of this surface in side view (angle not stated) with that of a practically perfect diffusing surface obeying Lambert's cosine law will very considerably increase the initial error.

Warburg and Negelein also reinvestigated P in this later work with somewhat different results. The functional test indicated that absorption was not complete in the red owing to the use of non-monochromatic light, some of which was of wavelengths where the absorption coefficient was relatively low. The test with a methyl alcohol extract showed a transmission of 3 percent at the two longer wavelengths, 10 percent at 546 $m\mu$, and less than 1 percent at 436 $m\mu$. The assumption was made that the transmission of the actual suspension would be less than this, and, especially in view of the fact that much of it would be re-

flected back by the silvered walls, that P could be neglected except, perhaps, in the green. It is not apparent why this determination of P should not have been made directly on a cell suspension and all assumptions avoided.

The Estimation of Absorption by the Green Pigments Alone

Up to the present, we have been considering the total absorption by the plastid pigments, both green and yellow. If we write:

$$A_p = A_g + A_y \quad (11)$$

for the separate absorption of these two groups of pigments, it is evident that in the longer wavelength portion of the visible, extending to about 540 m μ , we may consider A_p and A_g as identical. At some undetermined shorter wavelength, however, the yellow pigments also begin to absorb energy to an unknown extent.

For the interpretation of the data of photosynthetic function in light of shorter wavelengths, it is naturally of vital importance to know how much of the observed absorption is respectively attributable to the green and yellow plastid pigments. This has led to at least two attempts to interpret the absorption of the leaf in this region on the basis of the absorption of the extracted pigments.

The more extensive of these was that of Wurmser, who tried to give a complete interpretation of the absorption of the chlorophylloid pigments in *Ulva lactuca*. Somewhat remarkably, after pointing out the error of Timiriazeff in attempting to interpret the absorption of the leaf in terms of the absorption of the extracted pigments, Wurmser essayed to do this by subtracting the absorption coefficients of a petroleum ether solution of carotene from those which he found for the green thallus. The procedure was arbitrary in every particular. It was assumed that the subsidiary extinction maximum of the thallus at 492 m μ corresponded to the first absorption band of carotene at 480 m μ in alcoholic solution, the inflection at 460 m μ to the second carotenoid band at 450 m μ , and the principal maximum at 430 m μ to the carotenoid band at 425 m μ . The above mentioned absorption curve was then distorted to bring its maxima into these new positions and values assigned for the absorption coefficients to make the longer wavelength portion fit the thallus curve quite closely. These values were then subtracted from those obtained for the thallus, the difference being considered to be the absorption coefficient (K_g) of the chlorophylloid pigments. This procedure resulted in a practically flat absorption minimum, with $K_g = 0.02$ except at 560 m μ , extending from 590 m μ in the yellow to 460 m μ in the blue. This corresponds to an absorption factor of only 5 percent.

There would seem to be little justification for the large values assumed for the carotenoid absorption. The resulting low absorption coefficients for the chlorophylloid pigments in the green, blue, and violet are without doubt responsible for Wurmser's finding that, if the photosynthetic efficiency for the red region between 750 m μ and 560 m μ were taken as unity, the efficiency in the green region from 560 m μ to 460 m μ would be 4.00, and that in the violet region from 460 m μ to 375 m μ would be 2.35. The actual absorption of the green pigments must have been very much higher, especially in the green, than the values used in computing the efficiency¹¹.

Warburg and Negelein (1923) found that in the red, 4.4 quanta were absorbed per molecule of CO₂ reduced, and 4.3 quanta at 578 m μ , while at 436 m μ , 5.1 quanta were needed. The first two figures represent chlorophylloid absorption alone, while at 436 m μ the carotenoids are also absorbing. In an effort to find out whether this larger number of quanta required at this wavelength corresponded quantitatively to the added absorption of the yellow pigments, they made a determination of the absorption coefficients, at 436 m μ , of a methyl alcohol extract of the total cell pigments, and of the yellow pigments alone, after removal of the green pigments by saponification.

From the ratio of these two coefficients, it was found that in the alcoholic extract 31 percent of the total absorption was due to the yellow pigments. Applying this result to the quantum data for the cell suspension, it would appear that the green pigments got only 3.5 quanta out of the 5.1 absorbed. This led Warburg and Negelein to the statement that the yellow pigments apparently also functioned in photosynthesis, but less efficiently than the green ones.

It is of importance to realize that no quantitative statement whatever can be made as to the actual absorption of pigments *in vivo*, at any given wavelength, on the basis of the absorption coefficients of their extracts at the same wavelength. Not only is it probable that in some cases the *in vivo* pigments are altered chemically through extraction, but the form of the absorption curve of the extracted pigment is a complex function of the polarity and dielectric constant of the solvent, and of the electron configuration of the pigment molecule. A direct consequence of this is that a given solvent may not be expected to affect the forms of the absorption spectra of two different

11. In a later paper, (Ann. d. Physiol., 1:47, 1925) Wurmser has reported a utilization of 59 percent of the absorbed energy in the red from 700 to 590 m μ , and of 83 percent in the green from 590 to 490 m μ . While these values are less out of line than those cited above, they indicate that A_p , for the green, still has been relatively underestimated.

pigments in a similar fashion, or even to produce comparable changes in two absorption bands of a single substance, where these are related to the transitions of different electrons. Under these circumstances, the ratio found for the absorption coefficients of two dissimilar pigments at any particular wavelength is seen to be largely a function of the solvent selected, and it cannot possibly bear any simple relation to the ratio *in vivo*.

IV. The Analytical Approach to the Determination of A_p

From the foregoing it is apparent that attempts to estimate A_p from the extinction coefficients of green and albino, or decolorized, leaves are wholly unsound. It is also evident that, lacking a method of determining A_t , equation 9 is only of use for obtaining an approximate value in the regions of maximal pigment absorption, where T becomes zero, and A_t so small that it can be neglected, so that A_p can be considered equal to $1 - R$. Clearly a stalemate exists, and the only hope of escape lies in further analysis. In this section some of the more recent efforts in this direction will be briefly discussed.

Pokrowski's Analysis of R_s

In addition to his determinations of A , Pokrowski made an interesting attempt to express R_s as the product of two factors, S and A . The latter we will here denote by C , to avoid confusion with the absorption factor. S was assumed to be a function of the scattering power of the tissues, varying with the species, but only negligibly with wavelength. C , on the other hand, which appears to vary with wavelength, as some inverse function of the absorbing power, was assumed to be constant for any given wavelength regardless of the species. R_t , like S , was assumed to vary with the species but not with wavelength. Writing $R = R_t + SC$, Pokrowski assigned arbitrary constant values to R_t and S , for the leaves of the six species he investigated, and to C , for 13 wavelengths between 670 $m\mu$ and 480 $m\mu$, and calculated values for R . These were astonishingly close to those determined experimentally for this wavelength range, the difference being in all cases less than 0.011. As, however, the values found for R ranged from 0.175 to 0.034, the percentage disagreement was usually between 5 and 10 percent, and in one case 25 percent. Both the maximum absolute and percentage errors occurred at 670 $m\mu$ and 480 $m\mu$.

The value of these computations is much lessened by the arbitrary nature of the procedure. In order to obtain such good agreements in this range, which, it should be noted, does not include either of the main absorption maxima, it was necessary, in some cases, to assign highly unreal

values for R_t , such as, on the one hand, zero for *Fraxinus excelsior*, and, on the other, a value for *Tilia parvifolia* which was greater than the observed total of $R_t + R_s$ at longer wavelengths. It would seem likely that if values for R_t were determined experimentally and inserted in Pokrowski's expression, and the attempt made to extend the calculations to regions of higher absorption, the failure of the product SC to give adequate values for R_s would become much more apparent.

While Pokrowski's expression for R_s would not appear to be adequate, the fits obtained indicate that it is not without basis. From the analysis which has been given of the effects of various factors on the distribution of the entering flux ($P_0 - P_r$) it would seem indicated that, at any given wavelength (λ), the flux P'_s represents the net result of the continuous operation of σ , responsible for the eventual partition into P'_s and P , and of the absorbing power of the pigments and tissues in attenuating these fluxes.

This absorbing power is a complex function of the concentration and molecular absorption coefficients of various absorbing substances, of the pattern effect, and of the detour factor. To a large extent, therefore, it is a function of σ , which may be regarded as approximately constant for a given leaf, and of λ . In leaves with a high σ , P'_s increases at the expense of P , but some of this increase is offset by the effect of σ in increasing ω and, hence, the absorbing power. It must, furthermore, be recalled that this effect of σ on the absorbing power is also a function of the concentration and absorption coefficients of the absorbing substances, and that when these are high they operate directly to reduce ω in opposition to σ , but where they are low, the effect of σ on ω is of great magnitude.

Using the factor rather than the flux notation, Pokrowski's S to indicate the net partitioning effect of σ , and C' to represent a reciprocal function of the effect of the absorbing power on the rejected flux, we could write:

$$R_s = SC' (1 - R_t) \quad (12)$$

This, it will be seen, leads to the expression:

$$R = R_t + SC' - SC'R_t = R_t(1 - SC') + SC' \quad (13)$$

which differs from Pokrowski's equation in the important particular that the first term is now also a function of λ .

Naturally, with an additional degree of freedom in the first term, it should be easier to fit the experimental data over a wide range of wavelengths than with Pokrowski's equation, if values for S , C' , and R_t , are assigned in a purely arbitrary manner. However, as already suggested, it would seem possible to determine R_t separately,

and it should, therefore, be possible to submit equation 13 to an adequate test when the necessary data for R and R_1 become available. If it should prove possible to get consistent empirical values for S in this way, these may be further tested by means of the equation:

$$T = C (1 - R_1) (1 - S) \quad (14)$$

developed in the same manner as equation 13, C being the factor for the transmitted flux corresponding to C' .

V. The Absorption Spectrum of the Chromatophore

The concept that the determination of the absorption spectrum of the individual chromatophore would effect a satisfactory solution of the entire problem of the estimate of A_p , is a venerable one. It perhaps originated with Timiriacheff (1872), who apparently regarded the absorption spectrum of the extracted pigments as "normal", and identical with those in the plastid, and considered the "deformation" of their spectrum in the leaf to be due to an admixture of "white light", transmitted as a result of what we have here called pattern effect. Iwanowski (1907, 1913) modified this concept only to the extent of substituting a somewhat nebulous "reflection spectrum" for Timiriacheff's "white light". This was based on Vierordt's observation that R and T were not entirely similar in their spectral distribution.

In more recent years, Becking and Ross (1925) made a microspectrophotograph using a single *Euglena*. The densitometer tracing from this negative indicates no especial difference from similar uncorrected densitometer tracings from spectrophotographs of *Ulva*. Seybold (1932b) attempted an analysis of the distribution of the incident flux by the leaf, based on an estimation of the relative optical densities of the images of the plastids, and the surrounding cytoplasm, in photomicrographs of leaf sections. As the photomicrographs were apparently not made with monochromatic light, it is not possible to attach much significance to these density determinations on account of the fact that the plastids, cytoplasm, and the photographic plate, are all absorbing selectively in dissimilar ways. The method, however, would appear to have distinct possibilities for analysis, if monochromatic illumination were used. This was clearly indicated in microphotographs made some years ago, at the Hopkins Marine Station, in connection with a study of the thermal greening of brown algae, using quite narrow filtered regions in the green and orange-red.

It should be pointed out here that the frequent assumption that Lambert's absorption law can hold within the leaf is not warranted. The ap-

plicability of the laws of Lambert and Beer to leaves, has often been discussed. All too commonly it has been forgotten that both of these laws apply only to the absorption of monochromatic radiation, and, furthermore, that it is assumed that the absorbing substances are homogeneously dispersed in the medium through which the radiation is passing. In systems where the pigment is aggregated into discrete units, Beer's law can only hold where the variation in 'concentration' is represented by a variation in the number of 'discrete units' per unit volume, and not where the pigment concentration in the discrete units is varied (cf. Mestre, 1935). Moreover, in systems where scattering is present, Beer's law can only hold for the flux transmitted without deviation, which is negligible in the case of a leaf. Due to the effect of pigment absorption on ω , and hence on P , it is clear that Beer's law has no significance here. Owing to the utterly unhomogeneous distribution of the pigment, Lambert's law, likewise, is without application within the leaf, and to postulate it is to deny the entire histological picture. With multiple thicknesses of leaves, or algae, and a fully diffused incident flux, Lambert's law holds for extinction. That is to say, $E_n = nE_1$, where n is the number of leaves, and E_1 is the extinction for a single leaf.

The attack, by means of what may be called morphological analysis, presents interesting possibilities, but will only be discussed very briefly here. In this approach, the leaf, or thallus, is dissected and the optical properties of the different layers determined separately. Methods of this type have been found of value by the writer in the study of the transmission of such large brown algae as *Laminaria*, where almost all of the pigment is localized in a thin layer on either surface, and can easily be removed. Schanderl and Kaempfert have successfully applied this technique to the leaves of *Ficus elastica* and of *Cyclamen persicum*, and have arrived at a valuable picture of the transmission factors for the various layers. In order, however, to obtain a true picture of absorption, by means of this method of analysis, it will be necessary to make measurements not only of T but of R , for the various layers.

VI. Algal Thalli, Suspensions of Algae, and Cylindrical Plant Forms

In concluding this analysis of the absorption of radiation by leaves and algae, a few words must be said about the special problems arising in connection with the above mentioned special forms. The flat algal thallus would appear to be ideal material for spectrophotometric studies of pigment absorption. Successive samples of great uniformity can easily be obtained, there is no prob-

lem arising from the presence of air-cell interfaces, and surface effects are obviously less complex. With a collimated incident flux, R_1 will evidently be low, as the surface reflection is almost wholly regular, and n_2 and n_1 differ only slightly. In many thick forms, such as *Laminaria*, it is probable that R_2 will be very nearly equal to T , as the central tissues will completely scatter all entering flux, and both emergent fluxes will have traversed two substantially identical pigmented layers. In very thin forms, like *Monostroma* and *Ulva*, we may expect that R will be much smaller than T , and that the reflected flux will be more evenly scattered than the transmitted one. In the thalli of marine algae the value of σ is apparently largely dependent upon the state of hydration of the pectic substances of the middle lamellae.

The chief difficulty in the photometric study of the flat algal thallus arises from the fact that this material must usually be observed while immersed in water contained in a glass cuvette. This condition also prevails when working with suspensions of unicellular forms, or with water injected leaves, and, with the substitution of air for water, in the case of ordinary leaves enclosed in a respiration chamber. From the previous discussions of refraction and total reflection at glossy cuticular surfaces, and of reflection at the glass covers of thermopiles, it will be evident that correction must be made for the effects of the air-glass-water, or air-glass-air, interfaces of the containing cuvette or chamber, if serious error is to be avoided in the measurement of the diffused emergent fluxes. Up to the present no determinations of R seem to have been made on material observed under these conditions, and no determinations of T have appeared in the literature in which the above indicated corrections have been made. In his recent spectrophotometric study of the submerged algal thallus, Seybold (1934b) has found it necessary to resort to the use of arbitrary values for R , based on Shull's measurements for the leaf, in order to estimate A .

Suspensions of unicellular algae are of particular interest, owing to their wide use in connection with the Warburg-Barcroft technique of studying photosynthesis, and present certain distinctly characteristic optical problems. In general it may be remarked that due to the approximately spherical form of the suspended algae, the angular distribution of R_1 will be such that, owing to total reflection, only a small proportion of this flux will be able to find exit, through the surface of incidence, from the cuvette in which the suspension must necessarily be contained.

It will be evident that, in the case of suspensions, ω will vary with the cell concentration, as well as with changes in n_2 and n_1 . This depend-

ence of ω on cell concentration must be borne in mind when working with varying cell concentrations, since change in ω and change in pigment concentration, or in molecular absorption coefficient, are optically indistinguishable. The partition of the entering flux into transmitted and rejected fluxes will, of course, also depend on ω .

With decreasing cell concentrations, an increasing portion of the incident flux will pass through the suspension without contact with cells, and hence without deviation. For an additional consideration of this undeviated flux (P_u), reference is made to a paper dealing with suspensions of bacteria (Mestre, 1935). It should also be mentioned that pattern effects may become important if clumping of the suspended cells occurs.

For a consideration of cylindrical forms such as the coleoptile of *Avena*, or the sporangiophore of *Phycomyces*, reference is made to Neurnbergk, and to Castle (1933).

VII. Discussion and Summary

A general analysis of the distribution of a light flux incident on a leaf, leads to the conclusion that there are two unequivocal methods for determining the total energy (P_o) absorbed by the leaf. The first of these is the measurement of the total incident, transmitted, and reflected fluxes (P_o , P , and P') in a strictly comparable manner. The second is the measurement of the transmission factor ($T = P/P_o$), and the reflection factor ($R = P'/P_o$). The ratio of R to T increases with σ , the scattering power of the leaf, and also with the absorbing power of the leaf pigments and tissues. The absolute magnitudes of R and T depend, in part, on the nature of the leaf surfaces. They also decrease with increasing absorbing power.

It is indicated that σ is mainly a function of differences in index of refraction at interfaces in the tissues of the leaf, and that the absorbing power depends not only on the absorption coefficients and concentrations of the various absorbing substances, but also on two other factors. These are the detour factor (ω), representing the mean length of the light path through the leaf between incidence and emergence, and the pattern factor (p), which is a measure of the lessened effectiveness of the plastid pigments as absorbers, due to their being segregated into discrete chromatophores arranged in varied patterns.

While ω increases rapidly with increasing σ , and thereby proportionately increases the absorbing power, it is also, somewhat paradoxically, decreased by an increase in either pigment concentration or absorption coefficient. This has the important result of greatly increasing the relative absorbing efficiency of the plastid pigments in such regions of low absorption coefficient as the

green, or with low pigment concentration, as in young leaves or yellow varieties.

The optical characteristics of the leaf surfaces may not only be of importance in excluding incident flux from the tissues; but they may also function as a "light trap", partially preventing the escape of flux which has entered. The efficiency of a highly diffusing surface as a light trap is shown by the already cited observation of Shull that R for the lower surface of *Populus alba* only varies very slightly with wavelength. This clearly indicates that little of the entering flux is able to return through this surface. The leaf surfaces also play a very sizeable part in determining the angular distribution of P and P' , which is of considerable importance in connection with the measurement of these fluxes. Application of Fresnel's and Snell's laws to glossy surfaces indicates that their efficiency, both in excluding and in retaining fluxes, is probably larger than previously suspected, and that they may also have an important effect on the angular distribution of the emergent fluxes.

The infrared radiation from the leaf is of sufficient magnitude to be of importance in connection with the determination of the absorption factor, where thermopiles or bolometers are used for measurement, and would appear to be worthy of further study from the viewpoint of spectral distribution. The fluorescence emitted by the green pigments is of such very low intensity that it is quite negligible in the determination of P and P' . On the other hand, owing to the possibility, first stressed by Reinke (1883), that the ability of these pigments to fluoresce may be intimately related to their rôle in photosynthesis, it is a flux of extraordinary interest.

This interest has been much enhanced recently through the work of Kautsky and his collaborators, which has been reviewed in the paper by Taylor in this volume. As I remarked in the discussion of Taylor's paper, there would appear to be certain objections to a too ready acceptance of Kautsky's extension of his activated O_2 mechanism to include the photochemical reduction of CO_2 in the plant. These objections mainly arise from the fact that in all of Kautsky's work ultraviolet light was present, and because the amount of energy actually involved in the variation between maximal and minimal fluorescence is extremely small.

In nature, photosynthesis is, for the most part, carried on with energy derived from the longer wavelength portion of the visible through absorption by the chlorophylloid pigments. It is known, from the work of Warburg and Negelein, that photosynthesis can proceed with maximal efficiency in red light alone, and with slightly reduced efficiency in blue light. Nothing quantitative is known about photosynthesis in ultraviolet

light. but Ursprung (1917) has stated that it does proceed, although slowly. It is somewhat unfortunate that all of the work of Kautsky and his collaborators has, with the exception of a single experiment, been carried on with filtered ultraviolet light between the approximate limits 400 to 350 $m\mu$. As the light source used was a quartz Hg arc, irradiation was, presumably, mainly with the 360 $m\mu$ group of lines. In the single experiment mentioned, Kautsky (1934) has attempted to link the phenomena observed with ultraviolet excitation, more closely with photosynthesis by using the light from a carbon arc filtered through ammoniacal $CuSO_4$. No exact data are given as to the filter, or its transmission, other than the statement that the long wavelength visible was excluded, the transmission being practically limited to the violet and blue. The data of Wood (1934) show that a filter of this composition, having the stated transmission in the visible, would also transmit to about 350 $m\mu$ in the ultraviolet. In this case the possibility does not seem to be excluded that Kautsky's observed fluorescence effects are due wholly to excitation by near ultraviolet, and perhaps have no direct connection with the mechanism of photosynthesis in visible light.

Another fact pointing in this same direction, is the extremely low intensity of fluorescence in the leaf as compared with that of chlorophyll in solution. This leaf fluorescence is of such low intensity that its very existence was denied for many years, and it can only be observed with the aid of good filters and intense illumination. Kautsky himself (1935) has commented on the fact that only a very small fraction of the quantum absorbed ever appear as fluorescence in the leaf, even when it is maximal, and remarks that therefore the variation in fluorescence intensity can only be considered as an indicator of the course of the main transformation of energy of excitation into chemical energy. If, as Kautsky apparently assumes, the main alternative of the excited chlorophyll molecule lies between fluorescence and a transfer of the energy to his hypothetical labile oxygen compound, the fluorescence intensity, in the absence of O_2 , should rise to a very much higher level than that observed. Kautsky's data seem rather to point toward an assumption that the main transfer of energy is to some molecule other than O_2 , which is an essential link in the photoreduction process, and with which the chlorophyll is always closely associated. The transfer to the oxygen compound can then be regarded as representing a relatively minor reaction, which may, however, be important as indicating the existence of a photosensitized respiration, which is independent of the respiration occurring in the dark.

In regard to the often reported dependence of

photosynthesis on the presence of oxygen, it should be noted that there is evidence to show that this dependence is far from absolute. In Beijerinck's (1901) well known method for detecting photosynthesis, a small piece of an algal thallus is sealed in a tube with a thick suspension of luminous bacteria. If this preparation is then kept in darkness, the luminescence of the bacteria decreases and then ceases entirely, as the oxygen tension is reduced by their respiration, and by that of the algal material, to a level below the minimum required for luminescence. This minimum Harvey and Morrison have found (see Harvey, 1927) to be a partial pressure of 0.0053 mm. Hg., which would be only about 7×10^{-6} atmospheres. We may further calculate that this would correspond to about 2×10^{14} molecules of O_2 per cc. of suspension. If we assume that there are only 10^8 bacteria per cc., this would be 2×10^6 molecules of O_2 per bacterium. Taking Harvey's figure, that about 2×10^5 molecules of O_2 are required, per bacterium per second, for respiration, this would indicate that some ten seconds after luminescence ceased the culture would be practically anaerobic. If, when in this condition, a lighted match is held, for a few seconds, near the preparation, a distinct luminescence immediately follows, indicating that the almost absolute lack of O_2 has not prevented the initiation of photosynthesis. (cf. Molisch, 1905).

It would, on the whole, seem simpler to assume that the presence of oxygen has little direct relation to the photochemistry of CO_2 reduction, and that the frequently observed failure to initiate photosynthesis in the absence of O_2 indicates that the plant is dead, or nearly so. After all, we have no more right to expect a plant cell with an aerobic metabolism to survive intact when deprived of O_2 , than we would have in the case of an animal. In both plants and animals, it might be observed, there is evidence of a considerable variation in the ability to withstand reduced O_2 tensions.

A thorough recognition of the possible effects of physiological and pathological variations in plant material is of major importance in the determination of the absorption factor. Not only may differences in age, cultural condition, or genetic composition, be reflected in marked variations in surface, in σ , and in pigmentation, but extensive changes in p may take place during observation, if the plastids change their distribution pattern when irradiated. Pathological changes, whether preexisting, or resulting from the conditions of examination, must not be allowed to pass undetected, as errors of the first order of magnitude may easily result.

Many of these changes take place with the greatest rapidity, under certain conditions of ob-

servation, and their effect may then be offset by some other change producing an effect of corresponding magnitude in the opposite direction. An extreme, but striking, illustration of this is furnished by the optical changes occurring in brown algae dipped into hot water. At some wavelengths the initial great increase in T , which results from a decrease in σ , due to the rapid hydration of the pectic substances of the middle lamellae, is followed by an almost equally rapid and exactly equivalent decrease, due to swelling of the chromatophores with a resulting increase in p .

From a photometric viewpoint, it is apparent that there is no longer any reason why determinations, of a satisfactory order of accuracy, should not be made of A' , the absorption factor with a collimated incident flux. We have, however, reached a point where the continued accumulation, by a large variety of methods, of first approximation data concerning the energy absorption of leaves or algae, of an increasing number of species in somewhat indeterminate physiological states, would seem to be distinctly less useful than effecting a thorough-going general agreement on instrumental methods. Of these, two types appear to offer the best possibilities for the securing of accurate comparable measurements of R and T . These are Ulbricht sphere methods, as exemplified by Hardy's recording analyser, and mirror methods modified from that of Coblentz.

The different laboratory techniques should first be brought into as close harmony as possible by means of measurements on comparable material. This implies either that certain laboratories must be equipped to make such comparative measurements, with a variety of techniques on identical biological material, or that models of various sorts must be devised which may be passed from laboratory to laboratory for measurement or, preferably, both. This latter practice has proved of the greatest value to physical laboratories engaged with photometric problems, and there would seem to be no reason why biologists should not profit by it. It would appear to be entirely possible to construct adequate models out of glass cuvettes packed with fairly accurately sized green glass spheres, of a diameter of approximately 5μ , with the interstices variously filled with air, water, ethyl cinnamate, and other fluids if desired. Either or both of the cuvette surfaces could be ground, or etched with hydrofluoric acid, to simulate various leaf surfaces. It should, incidentally, be possible to learn much by the study of models of this type with varying n_2/n_1 and pigment absorption.

Where discrepancies in the measurements made with different instruments, or by different laboratories, were found to exist, these measurements should indicate changes in design, which would

bring about agreement, or at least make it possible to determine adequate corrective factors. The existence of standardized laboratory instrumental techniques would not only avoid much futile controversy, but should lead to the development of simpler, more rapid, and less expensive instruments suitable for use in the field and for many physiological studies. The utility of these instruments would be greatly increased by calibration against the more elaborate instruments.

The technique for determining A'' , the absorption factor for a diffuse incident flux, is evidently not in a satisfactory state. The determination of T for diffuse irradiation presents no difficulties, but, while it is quite probable that the corresponding determinations of R can actually be made, no strictly comparable method for doing so has yet been devised. Owing to the importance of A'' in ecological problems, and in laboratory experiments where growing plants are irradiated by a "cross-fire" from a number of artificial sources, it would seem to be quite worthwhile to determine the relation of A' to A'' with the greatest possible accuracy. As already suggested, it is quite possible that A'' , due to compensatory effects on R and T , may prove to differ very little from A' . If it does differ materially, it may still be possible to work out a factor for estimating A'' from a determination of A' , and of T for diffuse irradiation.

It is likewise evident that we are not yet able to determine A_p , the absorption factor for the plastid pigments. We can expect that in regions of high absorption, A_p will approach A rather closely, both becoming nearly equal to $1 - R$. When the pigment absorbing power is low, A_p will be less than A by a considerable amount, owing to the higher value of the tissue absorption factor A_t , resulting from the great increase in ω .

There would seem to be no point in attempting to extend the theoretical analysis further in the absence of adequate experimental methods and data. It is quite possible, as already suggested, that sufficiently accurate data may show many of the theoretically indicated effects to be of second or third order magnitude, which may safely be compensated for by approximate correction factors, or disregarded entirely. On the other hand, it must be borne in mind that only through genuinely precise photometric measurements will it be possible to decide such questions. For many purposes rather rough approximations are entirely sufficient, as other unknown variables of relatively large magnitude are present, but it should be realized that the ultimate goal of the photometric study of plant tissues is the development of the theoretical analysis and the experimental technique to a point where detectable differences

in the optical properties may be interpreted in terms of structure and physiological state, and *vice versa*.

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AN ANALYSIS OF ORIENTED MOVEMENTS OF ANIMALS IN LIGHT FIELDS

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In essence the "tropism theory" of Loeb¹ assumes that all oriented movements of animals result from the fact that differential stimulation of sensory receptors on the two sides of an organism produces a difference in the effective movements of the locomotor organs of the two sides. On the other hand, Kühn (18) advanced a classification which divided oriented movements into numerous categories on the basis of the paths taken by the animals in stimulating fields having different spatial arrangements, and assigned separate mechanisms to each category. This classification has had considerable acceptance and has been recently reconsidered in an extensive review by Fraenkel (12).

In the present discussion it will be shown that the various classifications of Kühn may be explained without making further assumptions than those implicit in the "tropism theory", at least with regard to orientation in which light is the stimulating agent. It will be necessary before proceeding to make a brief analysis of this general theory and define certain terms which we shall use.

Progression and angular velocities. Let us consider an organism moving in a horizontal plane by means of locomotor appendages distributed on two sides of a median perpendicular plane. If the effective movements of these appendages are equal in direction and magnitude on the two sides of this median plane, the animal will move in a straight line with a velocity v produced by the combined movements of the locomotor appendages; this we will refer to as the progression velocity. If, however, the effective movements of the appendages are not equal on the two sides of the median plane, the animal will continue to move forward, but will exhibit in addition an angular velocity ω about an axis located somewhere in the median perpendicular plane. Resulting from this rotation there will be a change in direction which for a given length of time will be equivalent to the addition of a vector component at right angles to the progression component. The arrangement of these velocity components is diagrammed in fig. 1 where the animal is assumed to be moving in the plane of the paper; AB is the median plane of the animal, a is the axis of rotation, ab is the progression velocity v , and bc is the lateral velocity component.

The path of the animal can be most conveniently described as the locus of the point a at the

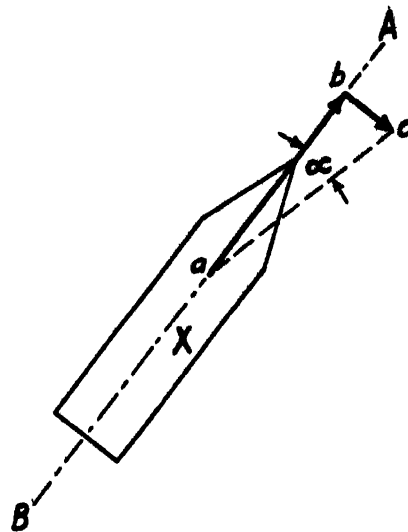


FIGURE 1

- AB = median plane of animal X.
- a = axis of rotation.
- ab = progression component
- bc = lateral component
- α = angle of turning.

intersection of the axis of rotation with the plane of movement. For a given interval of time, v will be measured by the distance travelled s , and ω by the angle α (fig. 1). Thus:

$$r/p = \omega/v = \alpha/s = K$$

The ratio K represents the curvature of the path of the animal at any point. If K has a constant value, the pathway will be a circle (see below); when $\omega = 0$, $K = 0$, and the path is a straight line.

If the animal has two or more sensory organs susceptible to stimulation by a field of stimulus, these organs must receive different amounts of stimulus, except when oriented in a particular way to this stimulating field. Although these sensory organs need not be bilaterally placed, for the time being it will be more simple to consider only this special case, and also to consider only the case of a parallel field of light rays. Under these conditions the sensory organs will only receive equal stimulus when the median plane of the animal lies parallel to the light rays; and if the differential stimulation of the organs exerts a differential influence on the effective movement of the locomotor organs on the two sides of the body, the animal will be subject to an angular velocity when it is placed in any other position. If the velocity of progression v remains constant, the curvature K then becomes a function of the

¹ This theory has been called by Mast (19) the Ray-de Candolle theory. We shall not be concerned here with the origin of this concept, and shall avoid the term "tropism" as it is in use in more than one sense.

differential illumination of the two receptor organs.

At this point the mathematical analysis of the problem becomes extremely difficult because of our lack of knowledge as to how the difference in stimulation of the receptors varies when the position of the organism changes with regard to the stimulating field. This should be some function of the angle which the median plane of the organism makes with the direction of the stimulating field. Fig. 2 illustrates a simple possibility.

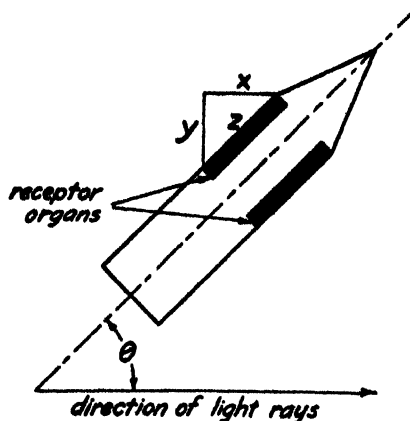


FIGURE 2

The dark surface z represents the photosensitive organ. The amount of light striking this organ will be proportional to y . $y = z \sin \theta$.

In this hypothetical animal only one sense organ can be illuminated at a time, and when the animal is oriented parallel to the light rays, no stimulation occurs. If we assume that the stimulation is proportional to the incident light, the angular velocity ω , will be proportional to the difference between the amount of stimulation of the two receptors:

$$\omega = f(y_1 - y_2)$$

where y_1 and y_2 are the quantities of light striking the two eyes. In the above case:

$$\begin{aligned} y_2 &= 0 \\ \text{and } y_1 &= k(\sin \theta) \\ \therefore \omega &= F \theta \end{aligned}$$

This particular case should be susceptible to mathematical analysis, but it would represent only a special case which probably never occurs in nature and is of no particular interest to us any more than innumerable other special cases. It is probable that in most instances the light strikes both eyes simultaneously and the function of θ then becomes a complex one depending upon the anatomy and physiology of the component units of the sense organs. Actually we can only say that ω is a function F of θ , the particular func-

tion F being different for every organism and probably not discoverable in the great majority of cases.

If our postulates are correct, the animal should move in a curved path until it is oriented with respect to the stimulating field, and at any instant the curvature will be a function of the angle which the median plane of the organism makes with the stimulating field.

The orientation-progression ratio. This would not seem very encouraging from the standpoint of quantitative study, but we may form certain general concepts which will, perhaps, be of greater value. Let us consider only the mean value of ω which we will call $\bar{\omega}$. Then:

$$\bar{K} = \bar{\omega}/v = a/p =$$

mean angular velocity/progression velocity

where K and a are the mean values of the curvature K and the angular displacement a , respectively. For convenience K will be referred to as the orientation-progression ratio.

A short consideration will reveal the importance of the orientation-progression ratio. If the value of K is great, which means that the rate of rotation will be rapid with respect to the rate of forward progression, the animal will align itself very quickly with the direction of the light rays. Once aligned, any tendency to deviate from a path parallel to these rays will be rapidly overcome, and the path will be very nearly a straight line. Animals which follow very exactly the resultant of the light field from two radiant sources must have a high orientation-progression ratio; they may be said to obey the "resultant law" and would be described by Kuhn's (18) system of classification as tropotactic. On the other hand, the path of an organism having a small orientation-progression ratio would approach the direction of the light rays in a gradual curve. Once aligned with the light rays, a deviation from a path parallel to these rays would be only slowly compensated and the animal would tend to follow a meandering path.

"Weak" and "strong" "tropisms". Thus we see that the path of the organism is quite as dependent upon the velocity at which it travels as upon the magnitude of the force tending to orient the animal. The terms "weak" and "strong" tropism have been frequently used to describe the accuracy with which an animal orients and remains oriented with respect to a stimulating field; and it seems that this is generally considered as dependent upon the magnitude of the orienting force produced by the stimulating field. From the above it is apparent that the path is really determined by the magnitude of both the force producing forward progression, and that producing orientation, i.e., the magnitude of the orientation-progression ratio. Thus a rapidly moving animal

must have a greater angular velocity than a slowly moving one in order to maintain its orientation with the same exactness.

Sign of orientation and sign of progression. Orientation is generally described as negative or positive. The difference in sign should depend upon whether the difference in stimulation of the sensory organs results in a greater or less effective tailward stroke on the side receiving the greater illumination. In the former case, whenever the animal is in a position other than parallel to the stimulating field, it will be rotated toward a position parallel with that field with the head away from the source of the stimulus—this may be denoted as a negative orientation; in the latter case the animal will be rotated toward a position parallel with the stimulating field with the head toward the source of stimulus—this we denote as a positive orientation.

It is necessary to distinguish between sign of orientation and sign of progression, since they may not always correspond, e.g., the lobster larva (2) and *Daphnia* (7); the organism may reverse the sign of progression without reversing the sign of orientation. Even if, in such cases, the mechanisms of orientation and progression are controlled by the same mechanism, as suggested by Blum (2) for the lobster larva, the signs of orientation and progression must be separately considered in describing the pathway of the organism. This is consistent with our method of analysis described. I will designate, therefore, progression in a headward direction as positive and progression in a tailward direction as negative. Fig. 3 indicates the scheme.

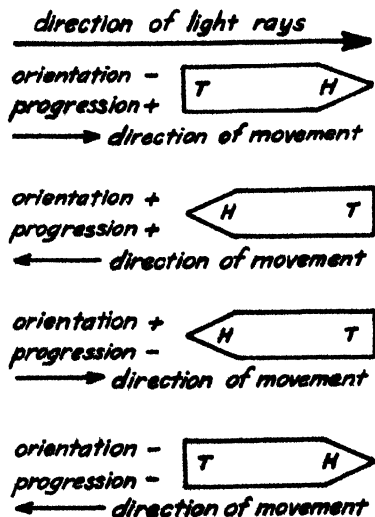


FIG. 3. H=head, T=tail.

Velocity and direction of movement. In our analysis of orientation we have regarded the velocity of forward progression as a constant value

for any given animal. If this were true the problem would be more simple than it really is. Actually, we find that most animals frequently change their velocity while proceeding in a light field. Assuming the angular velocity to remain constant, the curvature of the path must be altered with every change of velocity of the organism. This presents a great difficulty to any attempt to describe the path of an organism in terms of its angular and progression velocities. The only case in which changes of velocity will have virtually no effect upon the path of the animal is that of an animal having a very great orientation-progression ratio; in this case the animal will follow the path of equal stimulation and zero rotation very accurately.

Still another factor of great importance is the spontaneous alteration of the direction of movement. The existence of such change in direction is indicated in the observations of many investigators, for example, Mast (19), Spooner (23). We will not be concerned here with the cause of these changes in direction of movement, but assume them to be characteristic of the behavior of each particular organism.

TYPES OF ORIENTATION

With these points in mind we may proceed to a consideration of the various types of orientation described by Kuhn. The following is the classification as given by Fraenkel (11).

- A. Tropisms: oriented bending of sessile animals and plants.
- B. Taxis: oriented movements of motile organisms, particularly animals.
 - a. Phobotaxis: orientation in an undirected path (Ungerichtete Orientierungsbewegungen).
 - b. Topotaxis: movements directed in respect to a source of stimulation.
 - a) Tropotaxis: orientation in a stimulation equilibrium; if there are several sources of stimulation, the organism assumes a position on the resultant of the stimulation fields.
 - β) Telotaxis: orientation toward a goal; the animal moves directly toward one of a number of sources of stimulation.
 - γ) Menotaxis: orientation so as to maintain a definite angle between the direction of motion and a line joining the sensory organ with the source of stimulation. This

should result in spiral movements in radially directed rays from a point source.

- 8) Mnemotaxis: orientation to memory images.

It will be seen that with the exception of mnemotaxis the type of behavior characteristic of an animal should be detectable by the observation of the paths made under a few conditions of illumination, namely, (1) behavior in parallel rays, (2) behavior about a point source, (3) behavior in the field of two light sources. We shall proceed to consider the behavior of certain animals under these conditions.

Positively orienting animals. Tropotaxis and telotaxis. In the above analysis we considered only orientation in a field of parallel light rays. Every type of distribution of light rays presents a different geometrical problem. The combination of fields of light coming from more than one source may result in very complex stimulation fields; a simple example is the case of two parallel light fields meeting at an angle of, say, 90° in which an animal should be oriented toward the resultant of these two fields, i.e., should obey the "resultant law". The geometry of such a combined field does not change with respect to the organism as it moves about nor does the intensity of the light. However, when the animal moves in the field from two point, or small, sources the problem is more complicated since the directions of the light rays change with respect to the animal as it moves toward or away from the sources. Furthermore, the intensity at various parts of the light field varies, but for the time being we will assume that the light is sufficiently intense at any

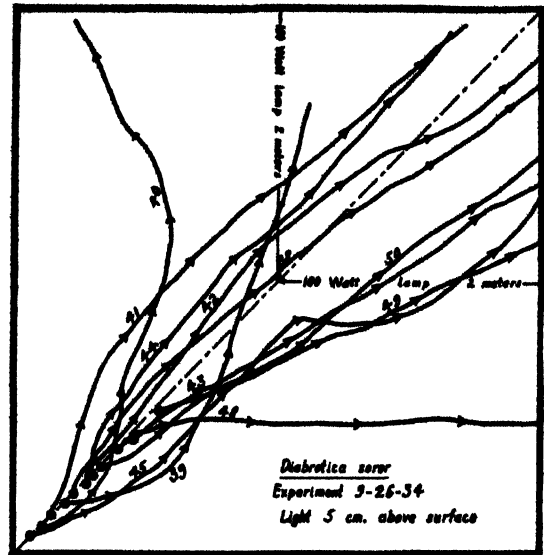


FIGURE 4

Experimental surface 50 x 50 cm.

point in the field to give a maximum stimulation to any photoreceptor unit. Thus the problem becomes merely one of the geometrical arrangement of the light rays and of the photoreceptor units.

Fig. 4² shows the paths followed by ten cucumber beetles (*Diabrotica soror*) in the field of light from two point sources placed two meters from

2 Figures 4, 5, 7-9, 11 and 12 are produced with the permission of the University of California Publications in Physiology, where the details of the experimental procedure used in observing the pathways will be published.

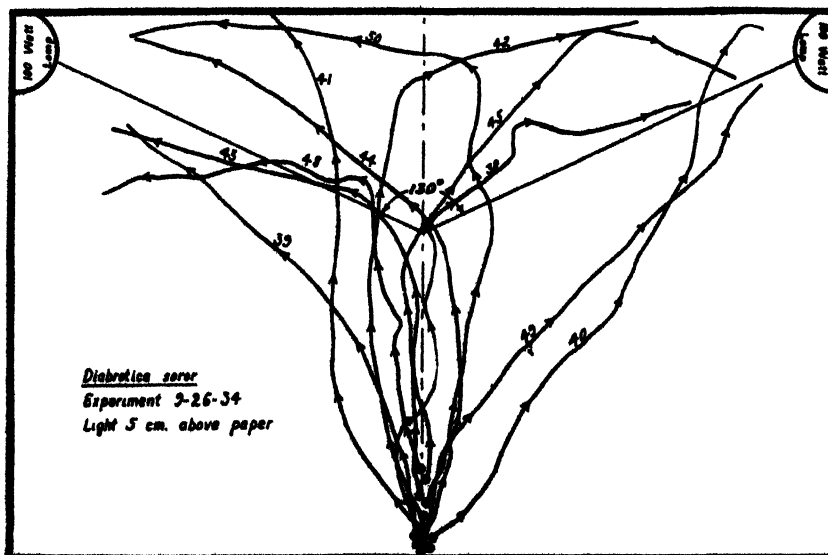


FIGURE 5. Experimental surface 50 x 75 cm.

the experimental surface and so arranged that they subtend an angle of 90° at the center of the surface. The majority of the beetles follow the resultant; thus the behavior is tropotactic according to Kühn's classification.

Fig. 5 shows the paths of the same individuals when placed closer to the same light sources, i.e., at a distance of approximately one meter. Some of the paths go quite directly toward one of the lamps (telotaxis), but the majority tend to follow the resultant for a distance before turning definitely toward one of the two lamps. A given individual does not behave consistently as either tropotactic or telotactic as will be seen by comparing figs. 4 and 5. If one examines the geometry of the eye of the insect, which is diagrammed in fig. 6, an

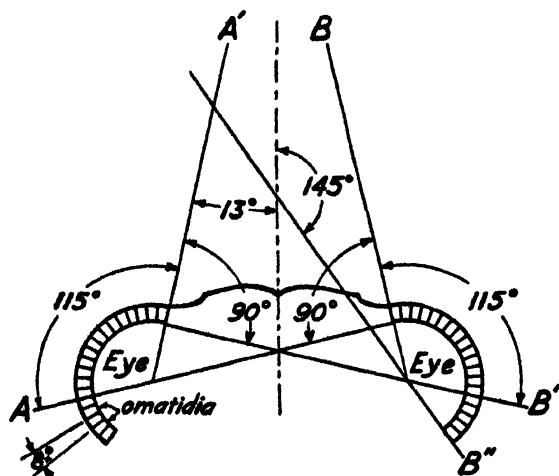


FIGURE 6

Diagram of frontal section of head of *Diabrotica soror*. For explanation see text.

explanation of this behavior appears. When the animal proceeds along the resultant toward the line joining the lamps, the subtended angle becomes greater, and when it reaches about 145° the condition is such that if the animal turns from the resultant so that its median plane coincides with the direction of one of the lamps, it will no longer receive a stimulus from the other lamp at any point on the eye. This is shown in fig. 6 for the lamp positions A' and B''. Reference to fig. 5 will show that many of the paths break away from the resultant at about this angle.

Fraenkel's (12) explanation of telotaxis depends upon the hypothetical arrangement and functioning of the receptor units in the eye, so that when certain units are fixed upon the image of the light source, deviation to either side results in stimulation of other receptor units which bring about differential movements on the two sides of the animal, thus tending to bring it back into position so that the image of the source is again

on the original receptors. There is certainly good general evidence for the existence of some such mechanism, at least in certain cases: (a) animals with one eye blinded, although at first showing circus movements or great deviation from a straight path, regain their ability to orient quite accurately to a light source (Holmes, 17; Minnich, 21; Mast, 20; Clark, 5 and 6), which indicates a certain special distribution of the impulses from the different ommatidia; (b) animals such as crabs may move sidewise toward a source of light which can only stimulate one eye; (c) flying insects may orient in a vertical plane, which must depend upon differential stimulus of parts of the eye and not differential stimulus of both eyes (Mast, 20). The detailed experiments of the above mentioned investigators give further convincing evidence that the response to stimulation of various areas of the eye is not uniform.

Actually this evidence may fit well with our analysis since it is only necessary to assume a differential stimulation of parts of the individual eye instead of differential stimulus of the two eyes. I have considered above only cases of bilaterally symmetrical stimulus in order to lend simplicity to our discussion and not to give the impression that other types of symmetry or even asymmetry of stimulus could not be explained. The geometry of such cases simply becomes more complex and the factors which we must know for an analysis, more difficult to obtain. I only wish to point out that the fact that an animal may move towards one of two sources may be explained on more than one basis and that the separation of such behavior into a distinct category is not justified, for the mechanism bringing about the same apparent behavior might be very different in two separate cases. Such factors as the size and distance from the source and the visual acuity of the animal are exceedingly important in the explanation of the behavior of the organism, but unfortunately these factors have not been taken into consideration by the proponents of the classification of telotaxis as a separate form of orientation.

The angle subtended by a single ommatidium of *Diabrotica* is somewhat over 8° (see fig. 6). This may be taken as an index of visual acuity for the insect, although the shallowness of the ommatidia suggests that the angle may be considerably greater. In our experiments the sources employed were 100 W. spotlight lamps, in which the luminous surface may be regarded as approximately one square centimeter. A source of this area will subtend an angle of only about $20'$ at a distance of two meters and does not subtend the same angle as a single ommatidium (8°) until the animal approaches within 8 cm. of the lamp. Thus under most conditions the animal may move through a considerable angle (at least 8° at two

meters distance) without changing the conditions of stimulation, i.e., without the stimulation of more than one ommatidium; thus the accuracy with which it can follow the resultant is distinctly limited.

The possibility of still further freedom of movement without changing the stimulus conditions exists. The simple arrangement of the om-

but on the above assumptions the stimulation conditions would be the same in each case, although the position of the beams differs by 115° . The fact that the animals behave so accurately should indicate that the results of stimulation of all ommatidia are not the same.

Menotaxis. Fig. 7 shows the paths taken by a number of cucumber beetles around a 40 W.

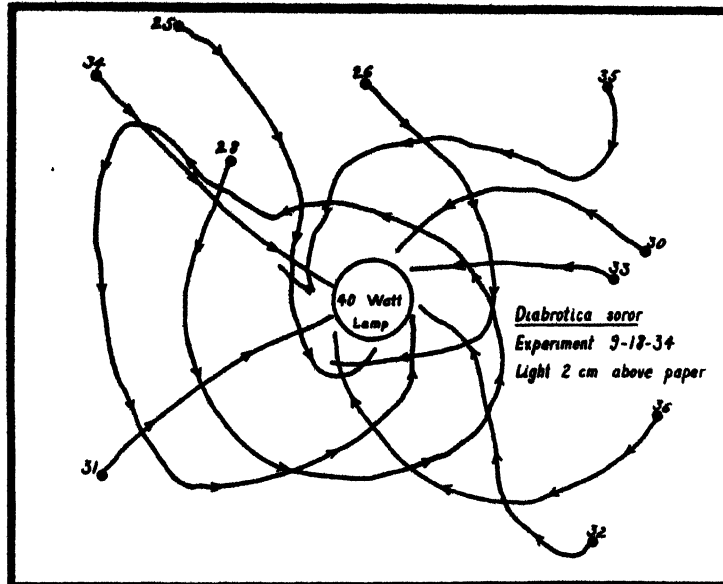


FIGURE 7. Experimental surface 50 x 50 cm.

matidia of *Diabrotica* on a spherical surface (see fig. 6) provides that a source of light will illuminate the same number of ommatidia at any angle within the limits of the spherical surface of the eye. If the intensity of the light is sufficiently great to stimulate any ommatidium it may strike (see Hecht, 15), and to elicit a maximum frequency of discharge in the nerve from that ommatidium (see Hartline and Graham, 14, and Hartline, 13), the number of impulses sent to the central nervous system should be approximately the same regardless of the angle which the eye makes with the direction of the source, within the limits of the spherical surface. Assuming further, for the purpose of argument, that the effective movement of the locomotor appendages is directly proportional to the number of impulses sent to the central nervous system, then the effect of stimulation of any ommatidium would be the same as stimulation of any other. We see by reference to fig. 6 that when the animal is placed so that the two sources subtend an angle of 90° , it may move through an angle of over 115° without changing the conditions of stimulation. For example, points A, B, and A', B' represent lamp positions such that beams strike the animal at 90° to each other; in both cases only one ommatidium can be stimulated by each beam,

Mazda lamp. The animals frequently move in spiral paths around a point source, though they move directly toward the source, perhaps more frequently. Figs. 8 and 9 show that the snail,

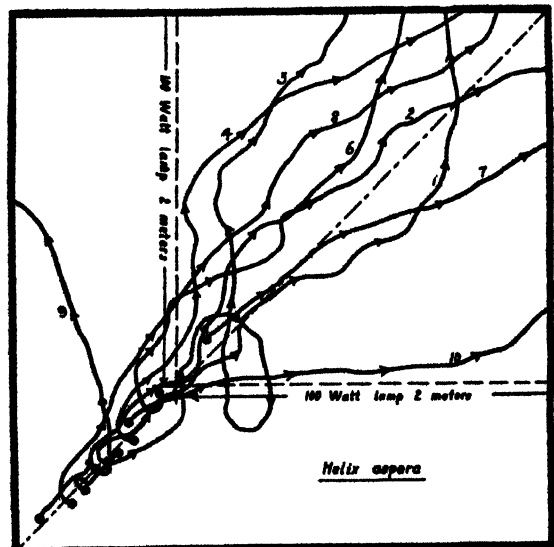


FIGURE 8
Experimental surface 50 x 50 cm.

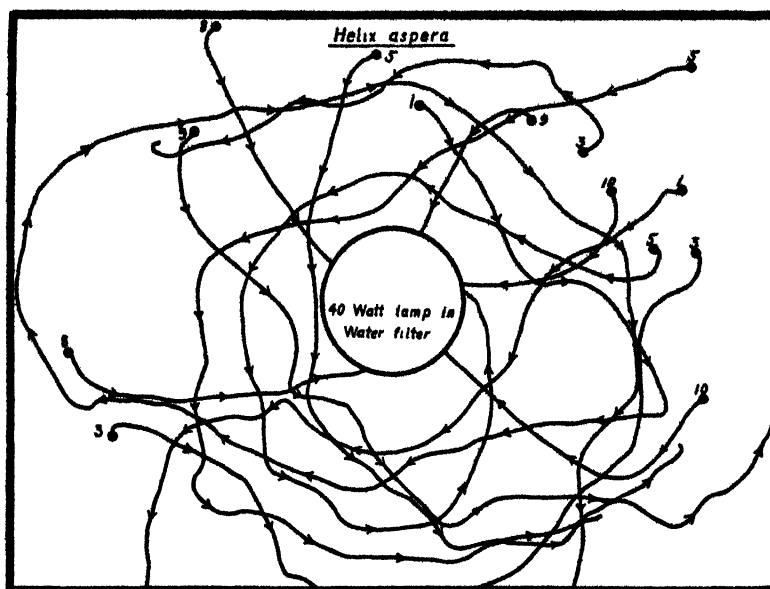


FIGURE 9. Experimental surface 50 x 65 cm.

like the cucumber beetle, may follow a light resultant (tropotaxis) under certain conditions and move in spiral paths under others (menotaxis). According to Fraenkel (12) an animal behaves menotactically when it moves so as to maintain certain sensory units of the eye in constant stimulation, thus holding the same angle with respect to the light rays. This was originally proposed by von Buddenbrock (3), according to whose analysis such behavior should cause an animal to proceed in a spiral path toward or away from a source of light. While such an analysis is quite reasonable, reference to figs. 7 and 9 will show that although *Diabrotica* and *Helix* tend to move in a spiral around a point source, they certainly do not hold the same angle with regard to the light rays. A careful examination of, say, path 28, fig. 7, and path 1, fig. 9, will clearly illustrate this (compare von Buddenbrock, 3, 4).

According to our analysis, an animal with a given rotation-progression ratio K might find itself so placed that it would travel in a circle about a point source. For every value of K there would be a given circle whose radius would be such as to satisfy the equation:

$$K = 1/R = a/s$$

Fig. 10 illustrates the arrangement of the lateral and progression displacement in this case. An animal finding itself with its median plane normal to the light rays at the proper distance from the source should tend to follow a circle of radius R , indefinitely. On the other hand, an animal finding itself aligned along the light rays would tend to move directly toward the source. Finding it-

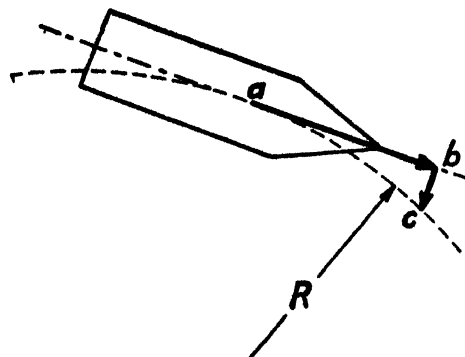


FIGURE 10

- a = axis of rotation.
- ab = progression displacement.
- bc = lateral displacement.
- R = radius of circle.

self at any other angle, the animal would be oriented toward one or the other of these paths, and in the latter case should describe some form of spiral.

Fig. 6 shows that if *Diabrotica* is placed so that the median plane of the animal forms an angle greater than about 13° (positions A' or B) with the direction of the light rays, it will receive light on only one eye, and should tend to take the circular or spiral type of path; if placed so that the median plane of the animal forms an angle less than 13° with the direction of the light rays, both eyes will be stimulated and the animal should move directly toward the light. It is clear from fig. 7 that these animals sometimes take a spiral path and sometimes a direct path toward the light.

If an animal following a circle in which $K = 1/R$ should change its value of K to K_1 by changing either its progression or angular velocity, it would tend to move toward the circle of radius such that $K_1 = 1/R_1$. In proceeding toward this new circle, the animal would move in a spiral course which would bring it toward the source if $K_1 > K$, but away from the source if $K_1 < K$. In the latter case we would find the apparently anomalous condition of an animal with positive sign of orientation and movement, travelling away from the light source. The flight of insects about a lamp often indicates such an occurrence; the animal may approach the lamp in a spiral course and as suddenly move away from it in a spiral course without any abrupt change in direction. This could be accomplished by simply changing the velocity of flight as well as by a change in the angular velocity; it is entirely unnecessary to invoke reversal of sign of orientation to account for this behavior.

Crozier (8) has suggested that spiral movement about a point source may be explained by assuming a vector toward the light source, which being a function of the light intensity decreases as the inverse square of the distance from the lamp; and another vector, not defined, which tends to maintain the animal in straight line progression. The similarity to our analysis is apparent, but the vector toward the source need not be assumed to vary as a function of the distance from the light.

Movement in circles or spirals about a source of light would, by our analysis, be similar to the circus movements of unilaterally blinded animals in undirected light. In both cases an angular velocity is produced because only one eye is stimu-

lated. Under the proper conditions the angular velocity should be a function of the light intensity for intensities producing less than maximal stimulation, as is clear from the experiments of Cole (8) on unilaterally blinded *Limulus*.

Negatively orienting animals. Tropotaxis and phototaxis. Fig. 11 shows the paths of ten in-

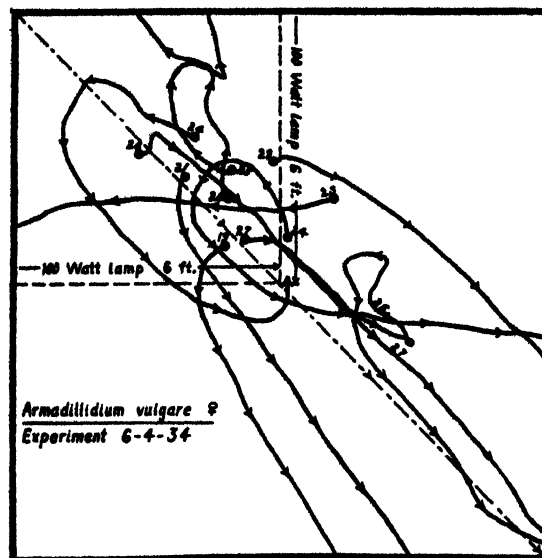


FIGURE 11

Experimental surface 46 x 46 cm.

dividuals of the species *Armadillidium vulgare*, the common pill bug, when placed in the same orienting field described for figs. 4 and 8. The majority of the animals follow the resultant with negative orientation. Fig. 12 shows the behavior

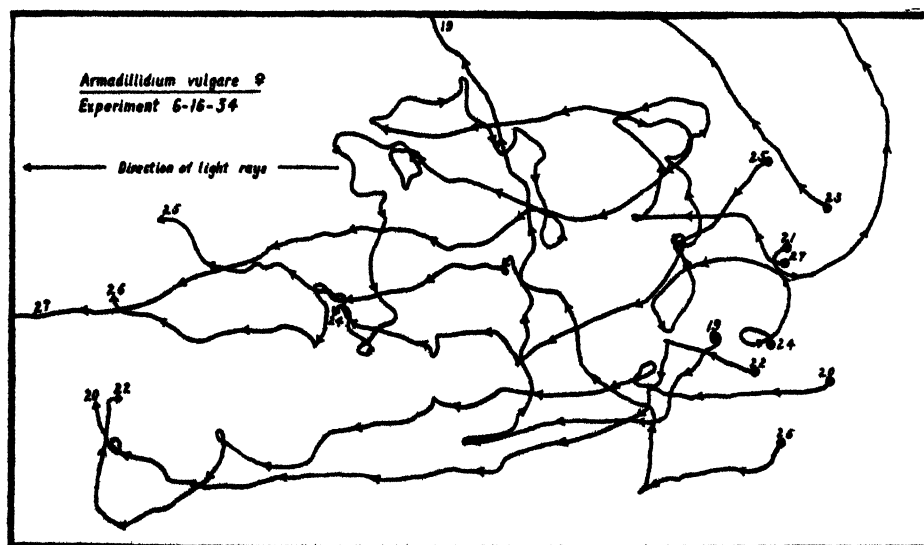


FIGURE 12. Experimental surface 48 x 85 cm.

of the same individuals in a field of parallel rays; here a great degree of randomness is exhibited. Difficulty arises in attempting to fit the behavior of these animals into Kühn's classification. In converging light rays these crustacea obey the resultant law and hence must be called tropotactic, but in parallel light rays they certainly show "orientation in an undirected path" (Ungerichtete Orientierungsbewegungen), if such a description is justified in any case; hence they must be called phobotactic. Thus Kühn's classification must again break down, since these animals follow two different types of behavior with different geometrical arrangements of the light field. On the other hand, the explanation which we have given above will fit both types of behavior with equal facility, i.e., in either case the animal may be considered as proceeding with a forward progression velocity which changes its magnitude and direction without regard to the light field, plus an angular velocity tending to bring the animal into alignment with the light field. Thus in parallel rays the animal wanders at random with a continual drift in one direction.

Reference to the geometrical arrangement of the eyes of this animal throws considerable light on its behavior. Fig. 13 may be accepted as show-

(positions A and B', fig. 13), and can thus be expected to display wandering, apparently un-oriented movement, to a great degree. However, in two light beams crossing at 90° , the animal may move only 125° without receiving a stimulus (positions A, B, and A'B', fig. 13). Reference to figs. 11 and 12 will show that the animal follows the light resultant with greater accuracy under the latter conditions. In diverging rays this angle must be still greater so that in the field about a point source *Armadillidium* may be expected to display an even greater amount of random movement.

A negatively oriented, positively progressing animal, when once aligned with a field of parallel light rays, with its head directed toward the source, would have no angular velocity and might be expected to move like a positively oriented animal. However, the probability of an animal retaining this orientation would be small. This is also true for such an animal in the field from two point sources of light when aligned along the resultant. In the case of *Armadillidium* we find that the single ommatidium of the eye subtends an angle of about 16° or more (fig. 13), and thus this animal when so placed might move through an angle of about 16° without changing the con-

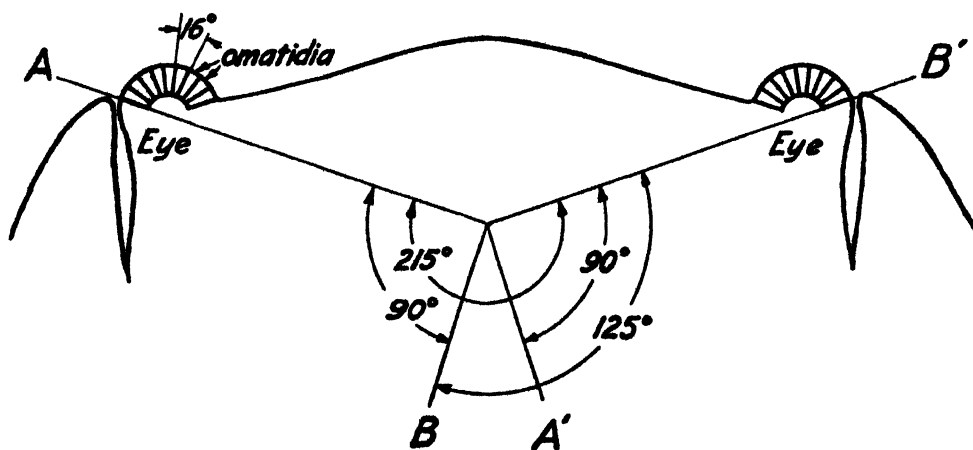


FIGURE 13

Diagram of frontal section of head of *Armadillidium vulgare*. For explanation see text.

ing the general arrangement of the head and eyes with sufficient accuracy for our purpose. Actually the detailed structure of the eyes was difficult to determine in our sections, and the angles given should not be considered as exact but as reasonably close approximations. Reference to fig. 13 will show that both eyes of the pill bug cannot be stimulated simultaneously by any light source or sources placed behind the animal. In a field of parallel rays *Armadillidium* may move through about 215° without receiving light on either eye

conditions of stimulation. It is thus not surprising to see animals behaving at times as though they were positively orienting forms (e.g., Nos. 20 and 25, fig. 11), and there is no need to assume that these individuals have temporarily changed their sign of orientation (i.e., reversed their tropism).

Kühn (18) and Fraenkel (12) place in the category of phobotaxis movements such as the "avoiding reaction" of *Paramecium* which are explained by them according to Jennings' concept of

"trial and error". It seems to the writer that random movement plus a drift due to the stimulating field working through a mechanism such as described above will account for this type of movement quite as well as "trial and error". In fact, the "trial and error" of Jennings and the "selection of random movements" of Holmes (16), contain within themselves the assumption that one direction of travel is facilitated over all others. Such facilitation must be due to the fact that in certain positions with regard to the stimulating field, the locomotor organs of one side move more effectively than those of the other; asymmetry does not affect this concept except in rendering the problem more complicated. Thus the fact that *Paramecium* and *Euglena* (see Mast, 19) display a particular type of movement because of their asymmetry does not free them from orientation by a stimulating field.

It may be noted that this type of movement is not limited to negatively orienting organisms but that it might be expected to be more pronounced in such animals; the term phototaxis would seem to recognize its predominance in animals moving away from a source of stimulation. On the other hand, menotaxis should never be found in negatively orienting forms, as appears to be the case.

Light intensity as a factor in oriented movement. We have simplified our problem in the above discussion by assuming that the light intensity remains constant as the animal moves about in the light field. This is, of course, only true for certain particular kinds of fields, e.g., parallel rays. It is necessary, particularly following the work of Hartline and Graham (14) and Hartline (13) to assume the all-or-none principle to hold for photosensory organs, i.e., light below a certain intensity is ineffective; actually the rate of discharge of impulses in the sensory nerve is a function of the intensity (see Hartline, 13), but this must also reach a maximum, so that we may assume that above a certain intensity further increase does not produce an increase in stimulation. Furthermore, the observations of Wolf (24) show a definite limit of intensity discrimination by the honeybee above certain intensities, as is also true for the human eye. This means that if the light intensity in all parts of the field is above a certain value, the field may be regarded as of uniform stimulation intensity, although the light intensity may actually vary greatly throughout the field.

In the experiments cited above we have employed light sources of relatively high intensity which probably provide a uniform stimulation of any receptor at any point on the experimental surface (the lamps used emit approximately 1000 lumens and the greatest distance from the source was 2.5 meters). Our experimental conditions were not free from criticism, e.g., in the case of *Diabrotica* and *Helix* there was a certain amount

of light reflected from the white surface on which the animals moved, but this should have been a relatively constant factor, and that it was is indicated by the fact that the few experiments which were performed on smoked paper gave similar results to those on white surfaces. Our experimental conditions approximate closely to those used generally in orientation experiments and thus our results may be considered as comparable. However, it must be pointed out that for studies justifying careful mathematical treatment many factors must be taken into account which have seldom been considered in orientation studies. For instance, many of the precautions to remove all extraneous visual objects are unnecessary because the objects are not within the visual acuity of the animal, but on the other hand, one must know the threshold of stimulus of the receptor organs and the way in which response varies with intensity before one can analyse the effect of changes in intensity upon the animal.

As stated above, in sufficiently intense light we may regard the stimulation field as constant and need only consider the geometrical arrangement of the sense organs and the direction of the light rays, but we must know the minimum intensity required to produce maximum stimulation of the photoreceptors before we can be sure of our ground. In less intense light the problem becomes much more complicated, for we must consider the relationship of the number of sensory units stimulated to the intensity of the light. The problem is further complicated by the phenomenon of light adaptation (see Clark, 5) which alters the threshold of stimulation. Studies of the type of the investigations of Hecht and his co-workers might be effectively combined with orientation studies in dim light, but the problem must be specially considered for each animal. The factors here considered, together with the geometry of the sensory organ, and the effect upon the motor organs of stimulation of various parts of this organ (see above) must all be components of the function *F*, discussed above, which must be known before the orientation can be described with any degree of mathematical precision. Thus it would seem that attempts such as those of Mitchell and Crozier (22) and Fraenkel (11) to predict the pathways of animals on the basis of the light intensity at various parts of the field could not be expected to meet with a great degree of success.

We will not have space to discuss mnemotaxis, a behavior which is probably so complicated that its explanation will not be greatly aided by our simple analysis. Neither will we discuss oriented bendings (tropisms) further than to point out that the problem is very similar, though somewhat simpler. Interestingly, the concept of spontaneous movement with drift would appear to find an analogue in the case of bending of plant

shoots, if we may accept the explanation given long ago by Charles Darwin (10) who assumed that the normal spiral growth movements (circumnutation) of plant shoots had superimposed upon them by light from one side, a component tending to bend them toward the light. Thus bending in one phase of the spiral would be affected more than in the opposite phase.

This concept of spontaneous movement with drift would seem to be extremely important with regard to the explanation of mass movements of animals, e.g., diurnal migration of plankton. It would be impractical to discuss its implications at this time but the writer hopes to do so in the near future.

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DISCUSSION

Dr. Wm. H. Cole: Blum's account of certain factors which must be considered in analyzing tropisms contains many excellent features. He has emphasized the necessity of knowing: (1) the geometry of the forces acting upon the orienting organism; (2) the threshold and maximum stimulating intensities effective on the organism; (3) the relationship between stimulating intensity (between those limits) and the rate or magnitude of orientation, and (4) the effective angle between the receptive surfaces. By illustrative experiments he has demonstrated how knowledge of these factors makes possible a logical interpretation of tropistic movements.

The introduction of the classifications of Kuhn and Fraenkel, however, seems to me very unwise. Limitation of the term, "tropism", to oriented movements of sessile organisms only; revival of the term, "taxis", long ago discarded; and the use of such terms as "phobotaxis", "topotaxis", "menotaxis", etc., will only tend to create confusion in the literature. Simplicity in terminology is just as desirable as it is in experimentation and interpretation. It would seem best for the present to avoid arbitrary terms which are only vaguely descriptive, and to postpone the invention of new names for special types of tropisms, until enough information has been accumulated to justify their use.

Dr. Blum: I am very sorry if I have not made myself clear with regard to the classification of Kuhn, and I wish to state that I feel that this classification has no experimental value and should be abandoned; except, perhaps, where it can be usefully employed for purely descriptive purposes. With regard to the use of the terms "taxis" and "tropism" I may say that while animal physiologists in the United States generally

use the term "tropism" in the sense of Loeb, i.e. to describe both oriented movements and bendings of sessile organisms, this use is not universal. As a rule European biologists, and botanists in this country use "tropism" to describe oriented bending, and "taxis" to describe oriented movement. I feel that the term "oriented movement" may well be used to describe both movement and bending; its use avoids any theoretical implications such as have come to be associated with the term tropism.

Dr. Wm. H. Cole: In his discussion of the subtended angle, Blum makes no mention of the so-called "head angle", H, as described by Crozier¹ (1925-28). Knowledge of the morphological angle between the photoreceptors is not alone sufficient, since the "head angle is the average effective angle between the bilaterally disposed receptive surfaces" and is determined by the physiology as well as the morphology of the receptor concerned, the stimulating intensity and "other conditions of temperature and concurrent geotropic excitation" (Crozier and Kropp,² 1934-35).

By devising simple experiments on a particular organism under a particular set of conditions and by applying rigid methods of analysis such as those of Blum and Crozier, satisfactory progress will be made in the study of tropisms. More and more complex situations involving the same organisms, and the behavior of other organisms, can then be studied with some hope of success. Gradually such terms as "trial and error", "avoiding reactions" and "random movements" will disappear from the literature.

Dr. Blum: In my discussion of the subtended angle I have used the morphological angle only as an approximate value. The "head angle" of Crozier, like my function F, includes the physiology of the receptor organs.

Dr. Clark: This paper by Blum gathers together certain significant facts of orientation. The demonstration of the relation between randomness of movement and visual acuity (e.g., the angle subtended by a single ommatidium), and the influence of the geometry of the eye upon orientation in light fields are cases in point.

With certain other phases of the analysis the writer does not have the same enthusiasm.

Blum in this preliminary theoretical analysis is correct for the simple case he sets up if it is assumed that there is a single receptor unit³ on each

side, or that there are a number of similar receptor units on each side, and the similarity is such that the response to a given stimulation of a receptor unit is qualitatively and quantitatively the same as the response to the same stimulation of any other receptor.

As far as is known to the writer there is not a single organism with receptors in accord with these assumptions. In those animals with a single receptor unit (*Euglena*, etc.), there is a single receptor. In those with symmetrically disposed receptors (*Planaria*, insects, etc.) the receptor organ is composed of more than one visual unit. And the units are not similar in any organism that has been studied critically (see Taliaferro 1920; Mast 1923; Yagi 1928; Dolley and Wierda 1929).

Other observations indicating differences in results of stimulating various ommatidia of the eye may be mentioned.

(a) By setting up good experimental conditions to give the "resultant effect" (see Blum Fig. 4) and assuming that (1) the same number of ommatidia at any angle within the spherical surface of the eye is stimulated to give the same number of impulses to the central nervous system; (2) the effective movement of the locomotor appendages is directly proportional to the number of impulses sent to the central nervous system, Blum shows that the animal should move through an angle of 115° without changing the conditions of stimulation. That accuracy of orientation is so much greater suggests that the results of stimulation of all ommatidia are not the same.

(b) In two beams of light of unequal intensities at right angles certain insects (*Eristalis*, Mast 1923) may orient so that one eye is illuminated by both sources and the other eye by one source only.

(c) Many insects with one eye covered will orient immediately or eventually and go directly toward a light source. If the ommatidia gave similar responses, orientation should not take place.

(d) In other cases the behavior of the animals in a light field can only be given adequate explanation by assuming the occurrence of such differential response (see Clark 1928, 1931, 1933).

Blum incorporates a factor for differential effect in his mathematical relation $\omega = F\theta$ in which ω (the rotation velocity) is a function F of the angle (see Blum fig. 2). Is it possible to give any indication of the elements that must be taken into consideration when an analysis is made of the actual response of animals?

From the works on insects above cited (Mast, Dolley and Wierda, and Clark) there is good reason to suppose that if only the inner ommatidia

1. Crozier, W. J., 1925-28, J. Gen. Physiol., 8, 671.

2. Crozier, W. J., and Kropp, B., 1934-35, J. Gen. Physiol., 18, 743.

3. In this discussion the single visual cell, visual ending or ommatidium will be called the receptor unit while the organ as a whole will be called the receptor.

of one eye are stimulated, a positive insect turns towards the unstimulated eye; if the anterior ommatidia are stimulated no turning occurs, and if the outer lateral ommatidia are stimulated the insect turns toward the stimulated eye. Furthermore the posterior outer lateral ommatidia when stimulated give a greater turning response than do the more anterior outer lateral ommatidia on stimulation. In other words there is a gradient in regard to the turning response from anterior to posterior of the eye. It should be noted that Yagi (1928)⁴ finds a dorsal ventral gradient in *Dixippus*. It may well be that any importance of the angle of the incident light is due to the fact that it indicates the ommatidia stimulated.

Dr. Blum: I must emphasize that the preliminary analysis given in my paper is entirely theoretical, and does not need the verification of finding an animal fitting the simple principles outlined. Probably none exists, and the value of the analysis is not altered by this fact.

I am indebted to Clark for additional examples indicating the difference in response obtained by stimulating different ommatidia.

Dr. Hecht: I should say that whereas most of us have been certain in our own minds that the classification used by Fraenkel was largely arbitrary and contained no real basis, it is rather a good thing to see quite experimentally without any preconception that the same organism, or similar organisms, behaves according to more than one of his types when placed under the proper conditions. The theoretical treatment is sufficiently general so that we need not take any exception to it, couched in such terms; one can modify the progression velocity and rotation velocity with intensity if he wants. This I think a legitimate procedure. Taking experiments and theory apart, which I think not an unreasonable way of treating your analysis, the thing which is significant is that you have shown that many of the variations in behavior are imaginary rather than real. The basic analysis Blum has made is, I think, probably sound.

4. The articles listed are in addition to those appearing in the paper by Blum.

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Yagi, N. 1928. Phototropism of *Dixippus morosus*. *J. Gen. Physiol.*, 11, 297.

Two things puzzle me a bit; the first one, the matter of the white surface, which I think Blum passed over too easily. If he is dealing with a point source which radiates in all directions placed 5 centimeters above the surface, a great deal of reflection would occur; the problem is not clear from that point of view. If you had a general light reflected which would maintain the animals in light adaptation, that would be all right, but by continuous light from below you may complicate your problem. I am a little surprised that the same thing happened when the animals were allowed to run on smoked paper. On general grounds I would be inclined to doubt that, and would expect more evidence before I drew a final conclusion. The matter of dark adaptation is something to consider more than you have.

Dr. Blum: We have realized the complicating factors introduced when the animals were allowed to travel on white surfaces. We began our experiments on smoked paper but had difficulty because the animals could not travel well on this surface, so we changed to the white surface, which gave in general the same results. It would be impossible to say that the results were exactly the same, because of the great variability of paths in both cases. According to my analysis the factors introduced by the white surface would fall into the function F, and would not obscure the general picture. This is probably the reason that the results with white and black surfaces are so similar. I may say, here, that the experiments and the analysis developed together. It is now possible to design a much better experimental treatment on the basis of our analysis. This we hope to do.

Dr. Hecht: Another point—you are not dealing with parallel light when you consider a point source at two meters distance.

Dr. Blum: Parallel light was used in only one case, that of *Armadillidium*. In the other instances, conditions were used which seemed similar to those employed by other investigators to demonstrate the types of Kühn. The analysis in those cases is not based on parallel rays.

Dr. Wald: Blum has analyzed the phototropic problem for the case of maximal stimuli, and assumed this condition to be satisfied in his experiments. I believe that this assumption is not supported by the available data. The work of Wolf (1933) on the bee, of Hecht and Wald (1934) on *Drosophila*, and the recent (as yet unpublished) measurements by Steinhardt on the human eye (Hecht, 1934) all agree in showing that at brightnesses up to more than 1000 millilamberts, intensity discrimination is still optimal, i.e., a minimal percentage increase in intensity produces a recognisable increment in stimulation. I think that the purposes of Blum's analysis

would be better served by substituting for this assumption the experimental device of placing the animal far from the source, so that reasonably small fluctuations in distance could produce little or no change in stimulation at any intensity. In fact, with such an arrangement, low intensities should be more desirable than high, since a given percentage change in intensity produces least change in stimulation in dim lights.

Dr. Blum: This would undoubtedly be a more favorable arrangement where it is possible to use it. However, in considering the behavior of an animal moving in a spiral path about a point source this would be difficult if not impossible in most cases. From a theoretical point of view the assumption of a maximum stimulating field is very useful as it reduces the problem to one of geometry and serves as a point of departure for experimental analysis.

Dr. Wald: Blum bases a considerable portion of his analysis upon the initial orientation of the animal with respect to the source. The argument should be greatly strengthened by observing and controlling this factor. The animals might be allowed to approach the source initially along a narrow, glassed-in track, which could be inclined at will to give them any desired initial orientation.

Dr. Blum: We have had such experiments in mind for the future. They should be very instructive provided we can avoid too much spontaneous movement.

Dr. Davenport: I am a little disappointed that Blum has been able to offer no new observations on the reaction of simple organisms to light. I

feel that we may learn a great deal about the action of light upon protoplasm by analyzing the phototactic reaction of, say, an amoeba to the incident light ray. In the higher organisms the light sets up a chain of reactions and in the course of the chain various events may occur that will alter the reaction. In some of the more complex organisms the same stimulus of light may cause now a positive, now a negative, reaction. What the reaction shall be at any moment may depend on the particular physiological state in which the organism finds itself.

Dr. Blum: I believe the general principles of our analysis would apply to both simple and more complex organisms. For example, I am quite certain we could demonstrate photo-orientation in man if we could design the proper experiment, just as we can demonstrate such behavior in lower organisms. I say this because I believe that the only requirement for orientation is a type of symmetry which provides that differential stimulation of receptors will produce differential movements of locomotor organs. This is what Loeb pointed out and I believe it remains the basis for all orientation—the modifications for each special case are a matter of detail. Such an arrangement provides for orientation simultaneous with other types of activity and movement. Thus an animal may drift in a given direction with relation to a stimulating field although this drift may be almost completely masked by other components of the behavior. This is why I say that spontaneous movement with drift is so important in the explanation of mass movements of the type found in nature.

PHOTIC EXCITATION AND PHOTOTROPISM IN SINGLE PLANT CELLS

E. S. CASTLE

Photic excitation of plant cells is taken to mean a change, induced by light, in some measurable activity of the cell. Photosynthesis and phototaxis are being discussed elsewhere in this symposium. The present discussion, therefore, will concern the effects of light on (1) cell growth and movement, (2) protoplasmic streaming, and (3) chloroplast movement.

Effects of the first type show the greatest resemblance to animal photoreceptor response; the last two types will be considered briefly only to call attention to certain interesting differences in the mode of action of light. In all of these cases it is necessary to be sure that photosynthetic effects are not involved, as they are, for example, in the operation of the stomatal guard cells. We are safe in working with fungus cells which naturally lack chlorophyll, or in growing potentially green cells in a chlorotic (etiolated) condition, or if chlorophyll is present in checking the spectral sensitivity of the process studied against the absorption spectrum of chlorophyll.

Most growth effects are fundamentally based on turgor. The plant cell wall offers a high resistance to the stretching force exerted against it by the cell contents. A slight change in the physical properties of the wall will permit the cell to expand or contract to a new volume, or to change one dimension at the expense of the others. Many cells are roughly cylindrical, and in such cases the extension of the wall may be unidirectional, parallel to the long axis of the cell and to the cellulose or chitin chains. The result is that the turgid cell may visibly react to a very small amount of radiation, if this acts to produce a substance affecting the properties of the wall. It is necessary to emphasize the sensitivity of such a mechanism, since at first sight a mechanical response might appear very gross.

Although the ultimate action of light in the case of all growth responses is on the cell wall, it is becoming recognized that this action is indirect, the primary absorption of light and a number of secondary events occurring in the cytoplasm of the cell. As far as we know, absorption of light by any photoreceptor cell is internal. The translation of an internal effect into a surface action brings up problems similar to those involved in the origin of nerve impulses in animal photoreceptors.

The absorption of visible light by cytoplasm must be mediated by some pigment present in small concentration, since cytoplasm is ordinarily regarded as "colorless". The fact that in all cases under discussion maximum sensitivity occurs in

the blue suggests that carotinoid pigments are involved.

I The growth response of *Phycomyces*.

The spore-bearing cell of this fungus exhibits the properties of its photochemical system more clearly than any other plant cell. It is essentially an upright, cylindrical, aerial tube with one end rooted in the substrate. Under constant external conditions the cell may show a steady rate of growth for some hours. A flash of light upsets this steady state, producing after a definite latent period a temporary acceleration of growth. (Fig. 1). Blaauw¹ states that he could detect a perceptible response after illumination of a sensitive

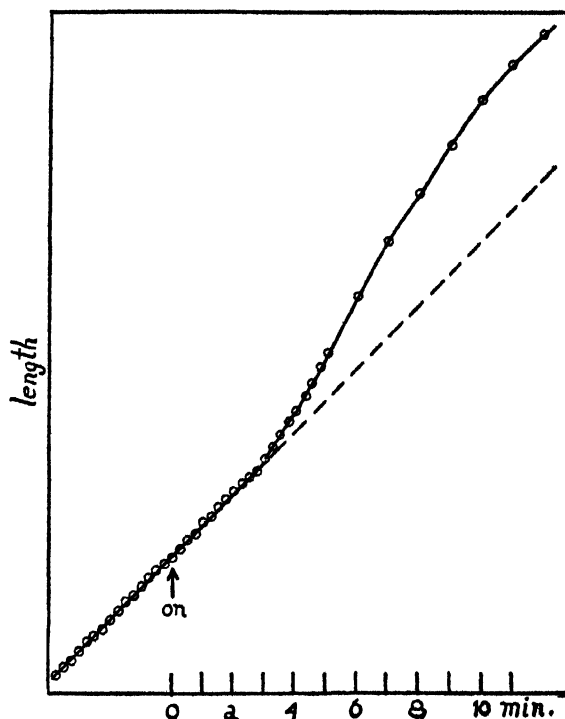


FIGURE 1.

The response of *Phycomyces* to a flash of light. Ordinate: length of the cell.

cell with white light of 0.01 meter-candles intensity for 1 second. While a strict conversion of these photometric measurements into energy units is not possible, the following rough calculation indicates the order of sensitivity.

¹ Blaauw, A. H., and Van Heyningen, W., *Proc. Acad. Sci. Amsterdam*, 28, 403, 1925.

Du Buy and Nuernbergk² estimated that for cells of the oat seedling, *Avena*, which like *Phycomyces* are most sensitive in the blue, 1 erg/cm²/sec. of wavelength 436 mμ was equivalent to an illumination of 25 meter-candles of white light from a tungsten lamp. On this basis

$$0.01 \text{ meter-candle} = 4 \times 10^{-4} \text{ erg/cm}^2/\text{sec.}$$

In *Phycomyces* the photosensitive region is a cylindrical zone about 0.01 cm. in diameter and 0.15 cm. long. Its surface is therefore

$$\pi \times 0.01 \times 0.15 = 4.7 \times 10^{-3} \text{ cm}^2.$$

If this surface is illuminated from all sides with an intensity of 4×10^{-4} erg/cm²/sec., then in one second, assuming that 10 percent of the radiation is absorbed, we get absorption amounting to

$$4 \times 10^{-4} \times 4.7 \times 10^{-3} \times 10^{-1} = 1.9 \times 10^{-7} \text{ erg.}$$

While this estimation involves a number of guesses, it does show that the energy necessary to produce a perceptible mechanical response in the cell of *Phycomyces* is extraordinarily small.

The substance which absorbs the effective radiation is almost certainly a carotinoid pigment. A pigment having an absorption spectrum much like that of α -carotene can be extracted from the cells by petroleum ether. Its spectrum is matched reasonably well by the data on the phototropic sensitivity of *Phycomyces*. (Fig. 2). It is interesting that recent work of Johnston³ on the phototropic sensitivity of *Avena* shows maxima at 440 and at 470-480 mμ, as compared with maxima at 449 and 475 mμ for the *Phycomyces* pigment in hexane.

Exposure of the cell to continuous light is found to decrease enormously the sensitivity of the response to a given flash of light^{4,5}, and this loss of sensitivity is some function of the incident intensity, as with photoreceptors in general. Changes in the level of adaptation to light are reversible, and it is clear that they represent changes in the concentration of a light-sensitive substance which is built up in the dark. The transfer of a cell from continuous light to darkness is followed by an orderly increase in sensitivity, the process of dark-adaptation. Figure 3 shows the course of dark-adaptation as judged by decrease in the reaction time of the response

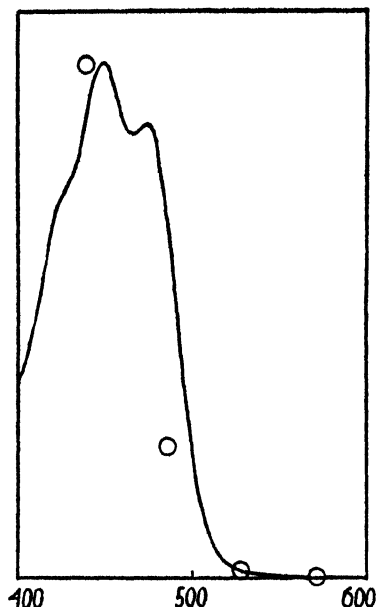


FIGURE 2.

Solid line: the absorption spectrum in hexane of a carotinoid pigment extracted from *Phycomyces*. Circles: spectral sensitivity data for *Phycomyces*. Abscissa: wavelength in mμ.

to a given flash of light. It is approximately complete within 30 to 40 minutes. Although the new level of adaptation has been reached in the photosensitive system within 40 minutes, the data of Oort⁶ show that other parts of the response mechanism are not restored so rapidly. After a response has occurred, as much as two hours may be required for the growth processes to build back to the initial steady state.

Thus far we have seen that the properties of the light system in *Phycomyces* are essentially similar to those of the more familiar animal

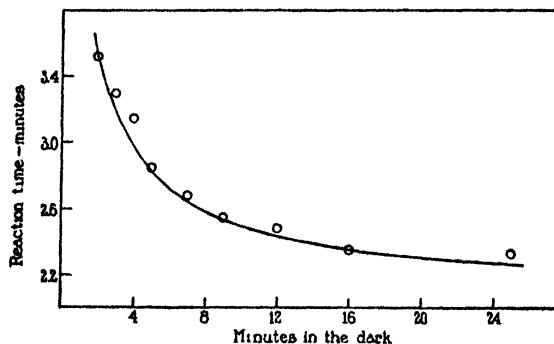


FIGURE 3.

The course of dark-adaptation in *Phycomyces*.

² Du Buy, H. G., and Nuernbergk, E., *Ergeb. Biol.*, 10, 207, 1934.

³ Johnston, E. S., *Smithsonian Misc. Coll.*, 92, No. 11, 1934.

⁴ Tollenaar, D., and Blaauw, A. H., *Proc. Acad. Sci. Amsterdam*, 24, 17, 1921.

⁵ Castle, E. S., *J. Gen. Physiol.*, 12, 391, 1929.

⁶ Oort, A. J. P., *Verhandl. k. Akad. Wetensch. Amsterdam*, Sect. 2, 29, No. 3, 1932.

photoreceptor. The nature of the end response is of course very different, and there also exists a striking difference in the duration of the latency. Plant cells generally show slower responses to light, and the reaction time of *Phycomyces* under most favorable conditions is at least 2 minutes, and may be as much as 10 minutes. Practically all of this is latency, and we find, moreover, that only the light which strikes the cell during the first few seconds of illumination is effective in speeding up or magnifying the response. Figure 4 shows how short the effective

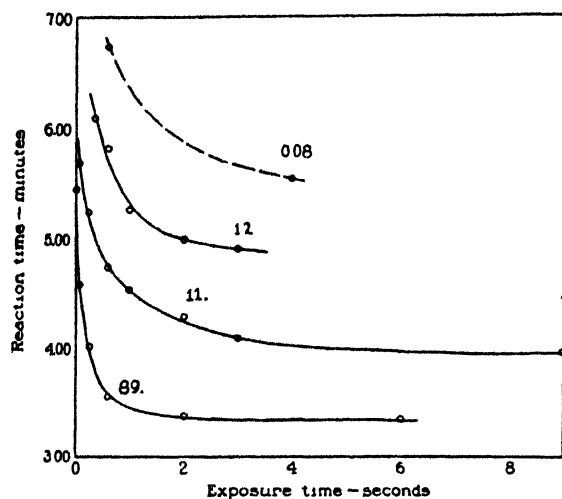


FIGURE 4.

Reaction times of *Phycomyces* with different intensities and durations of exposure to light. The intensity for each of the four curves is given in foot-candles. (Castle and Honeyman, *J. Gen. Physiol.*, 18, 385, 1935).

summation interval for the action of light is. After 3 to 4 seconds of exposure the reaction time is unaffected by further illumination, even though the response does not occur until 3 or 4 minutes later. This seems to be an exaggerated case of what Hartline⁷ detected as a "critical duration" in the action of light on the photoreceptor of *Limulus*. He found, for example, that for a particular intensity, the maximum frequency of impulse discharge is obtained with exposures to light of only 0.1 second, even though the impulses do not begin to appear until 0.259 second. In the case of *Phycomyces*, the short summation interval must mean that secondary processes, occupying the latency, begin the moment any of the photochemical products appear. We may suppose that a diffusion or reaction wave gets under way with a velocity determined by the initial concentration which is

set up. After a few seconds the wave front will be out of reach of new products of light action, and its steepness therefore unaffected by longer exposures. On this basis we might expect the effective summation interval to be shorter for higher intensities, and the curves of Fig. 4 suggest that this is true. It should be noted that in no case do we deal with a complete exhaustion of a fixed amount of light-sensitive material, since the curves for the different intensities descend to separate base-lines.

It is hard to avoid thinking of the secondary processes of excitation in terms of spatial translocation of a product or effect of light action to the wall. That the primary action of light is cytoplasmic seems clear from the response of the cell to γ -radiation¹. The effect here is a reversible inhibition of growth, occurring after a similar irreducible latency of 2 minutes. While a direct action of γ -rays on the wall would not be surprising, the delayed action points to the intervention of just such a protoplasmic factor as we have postulated in the case of the light response. Both visible light and γ -radiation, although producing opposite effects on the growth system, must have to do their work through the same train of machinery, which one accelerates, the other inhibits. Furthermore, narcotics such as ether vapor, or replacement of air by nitrogen, promptly reduce the rate of growth of these cells to a few percent of the initial rate. Although turgor is maintained, a flash of light produces no growth response. These facts also indicate an indirect action of light. Since nerve impulses make their appearance as surface phenomena, it would be interesting to know if the idea of translocation, which need not be synonymous with diffusion, has any validity for the more typical photoreceptor cell.

II Action of light on protoplasmic streaming.

Active circulation or streaming of cytoplasm occurs normally within practically all plant cells. Study of the action of light on this process is handicapped by the fact that in spite of one hundred and fifty years of intermittent research the mechanism of the movement is unknown. Recent observations of Bottelier⁸, however, on the velocity of streaming in *Avena* cells as affected by flashes of light are of interest. His work not only tells us something about the mode of action of light, but makes it very probable that in the oat seedling the streaming controls the transport of growth hormone. The cells which were used were free from chlorophyll, so that there is no confusion between photosynthetic and other effects.

⁷ Hartline, H. K., *J. Cell. Comp. Physiol.*, 5, 229, 1934.

⁸ Bottelier, H. P., *Rec. trav. bot. néerl.*, 31, 474, 1934.

The effect of a sudden flash of light is to cause a temporary inhibition of the rate of streaming, after a lag of 3 to 4 minutes. (Fig. 5). Within

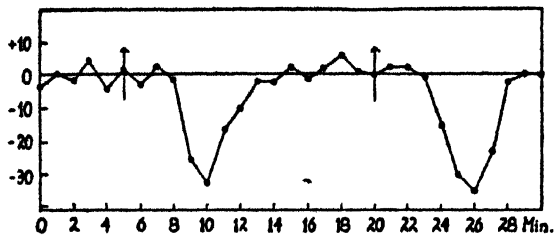


FIGURE 5.

Velocity of protoplasmic streaming in cells of *Avena* as inhibited by flashes of light. The occurrence of a flash is indicated by a vertical arrow. Ordinate: percent deviation from the mean rate of streaming. (Modified from Botteller⁸).

12 minutes the original rate of streaming has been regained, and within 20 minutes recovery is complete, that is, another flash will produce a second inhibition of equal size. To produce a response of a given size it is found that the Roscoe-Bunsen law holds satisfactorily with exposures of up to 3 or 4 minutes duration. In other words, summation is effective within practically the whole of the latency. Du Buy⁹ found essentially the same relation for phototropic bending in *Avena*. The case of *Phycomyces* is in striking contrast, the summation interval there being only a few seconds. In view of such findings, the early work on the phototropism of these plants which stated that time and intensity were strictly interchangeable over an enormous range (exposures up to 1 or 2 days) must certainly be wrong. A just perceptible degree of curvature is a very uncertain thing to measure. These experiments should be repeated using as an endpoint a curvature of definite size.

Beyond a certain point, Botteller finds that higher intensities of light give smaller inhibitions of streaming. High intensities may even accelerate streaming. This effect is obtained with carefully filtered monochromatic light, and must be genuinely photochemical. It seems to indicate a dual mechanism in the response, and fits in strikingly with changes which occur with increasing intensities of illumination in the light-growth responses of the oat coleoptile as a whole.

The spectral sensitivity of the effect on streaming is greatest in the blue. (Fig. 6). This finding coincides with data on the phototropic sensitivity of *Avena*, and indicates that here also we have absorption by a yellow pigment. Although adaptation has not been thoroughly studied in

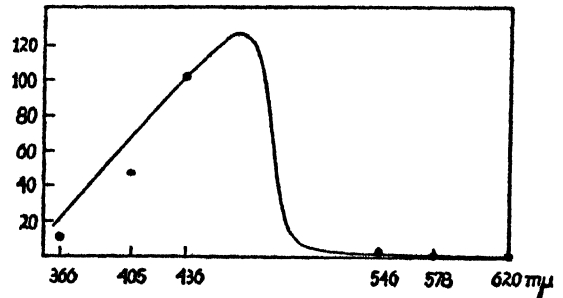


FIGURE 6.

Spectral sensitivity of effect of light on streaming in *Avena* cells (solid circles). Solid line: phototropic sensitivity curve of *Avena* according to Blaauw. Ordinate: relative sensitivity. (Modified from Botteller⁸).

this system, it is clear that susceptibility to repeated light stimuli means that recovery takes place. Aside from the special nature of the end-response itself, the principal deviation from the type of mechanism in *Phycomyces* seems to lie in the prolonged summation interval.

III Action of light on chloroplast movement

The location or arrangement of the chloroplasts in many plant cells is determined by light. Different plants may show different types of response. The forces producing movement of the plastids are unknown, but the response to light is definite and possesses some unique features.

Voerke¹⁰ has recently investigated the response in the leaf of the moss *Funaria hygrometrica*. Each cell contains from 15 to 30 chloroplasts which in the dark lie appressed to the side walls which are in contact with other cells of the leaf. In this state the leaf is relatively transparent. In the light the plastids move out and cover the exterior wall nearest the light, thus exposing a much larger absorbing surface. When the light is turned off they return to the "dark" position. The onset of these migrations is not sharp, perhaps with a latency of a few minutes. The whole process occupies 30 to 40 minutes.

An interesting feature of the response is the relation to intensity. Measuring the percent of the plastids in each cell which migrated to the "light" position, Voerke obtained for blue light the intensity-effect curve shown in Figure 7. Each intensity produces a definite, sustained action which is independent of time. This continuous response must be due to a balance between the light effect and a force acting to restore the plastids to the "dark" position. Since the plastids contain chlorophyll, it might appear that the light action is based on photosynthesis. Yet this cannot be true, since the spectral sensitivity of the plastid movement is greatest in the

⁹ Du Buy, H. G., *Rec. trav. bot. néerl.*, **30**, 798, 1933.

¹⁰ Voerke, S. H., *Planta*, **21**, 156, 1933.

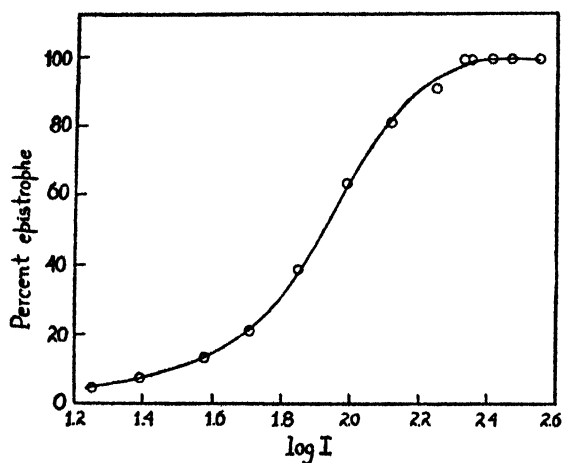


FIGURE 7.

Percent plastid migration (epistrophe) in cells of *Funaria* as a function of intensity of continuous blue light. Abscissa: $\log (\text{cal./cm}^2/\text{hr.} \times 100,000)$. (Data of Voerke¹⁰).

blue, and red light is wholly ineffective in evoking it.

It seems, then, that this is one more case of an excitatory action of light based on "cytoplasmic" absorption of the shorter wavelengths¹¹. The nature of the response conceals the phenomena of adaptation which are so prominent in other systems. It should not be thought, however, that in other plant cells the response to light is wholly discontinuous. For instance, in the case of *Phycomyces* steady illumination produces a comparable sustained action on the growth system, but this is made evident only by the dark-growth response which occurs when the light is cut off¹². In these moss cells we have visible evidence of the continued action of light.

IV Phototropism

Since phototropism results essentially from an asymmetric action of light, phototropism in single cells is merely a special case due to unequal internal distribution of light. With an incident beam of parallel light, such inequalities may be caused by refraction within the cell or by rapid absorption which reduces the intensity reaching the far side.

The clearest case of the rôle of refraction in causing phototropism of single cells is that of

Phycomyces. Only brief mention need be made of the analysis of this case, since it has been published elsewhere¹³. If illuminated from one side in air, the cylindrical cell grows toward the light. As we have seen, the action of light on this cell is to accelerate growth, therefore the light effect must be greater on the side of the cell farther from the light source. Refraction serves to concentrate light on the back wall, but the total luminous flux passing through the back wall must, as a consequence of absorption within the cell, be less than that passing through the front wall. (Fig. 8). The apparent paradox of greater light action in the back of the cell is understood if we

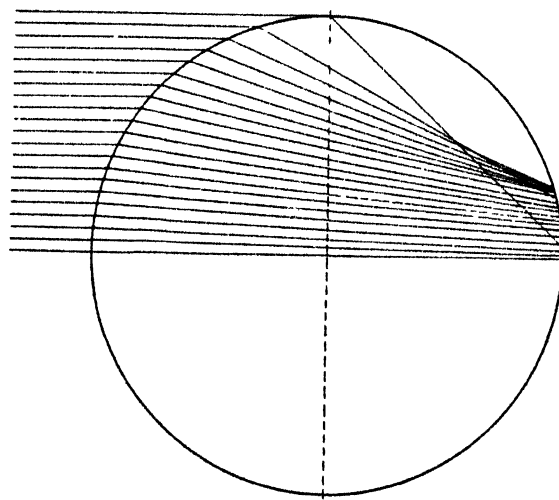


FIGURE 8.

Diagrammatic cross-section of a cylindrical cell in air, to show effect of refraction on length of absorbing path in the two halves of the cell.

suppose that light is absorbed throughout the cell, and secondarily produces its effect on the wall. Measurement of the total absorbing path in the front and back halves of the cell shows that as a consequence of refraction the total absorbing path in the back half may be more than 25 percent longer. This difference is able to overbalance the loss of intensity suffered by light rays on their way through the front half of the cell, with the result that the net absorption in the back half is greater.

Within single cells, asymmetric action of light due to refraction seems to be the most important factor in phototropism, although these effects may be outweighed by rapid absorption in the near half of the cell. Perhaps the most striking dem-

¹¹ Data on the spectral sensitivities of a number of other plants are given by H. G. du Buy and E. Nuernbergk, *Ergeb. Biol.* (in press). I am grateful to Dr. du Buy for allowing me to examine the manuscript of this part of their monograph.

¹² Castle, E. S., *J. Gen. Physiol.*, 16, 75, 1932.

¹³ Castle, E. S., *J. Gen. Physiol.*, 17, 49, 1933.

onstration of the influence of refraction is that of Buder¹⁴, who obtained negative, i. e., reversed, phototropic bending of cells of *Phycomyces* immersed in paraffin oil. On the other hand, penetrating radiation which is not refracted, such as γ -rays, produces no phototropism whatever¹. Other polar effects of visible light, such as the determination of the first cleavage plane of certain plant spores, are probably based on refraction.

CONCLUSION

In this discussion I have intended to call attention to an interesting class of light effects discovered long ago but not well known to physiologists generally. The action of light in these cases is not a synthetic one, and is independent of the presence of chlorophyll. In every case, however, a carotinoid pigment appears to be present. In serving to release rather than to store energy, these effects of light have most in common with excitation in animal photoreceptor systems. In some cases, as in *Phycomyces*, the phenomena of adaptation are prominent, and can be understood in terms of a reversible photochemical system such as seems to underlie the great majority of light responses. In other cases, the processes of adaptation are masked by the slow nature of the response, and we can only observe the steady state set up by the new intensity, as in *Funaria*.

The visible effects of excitation which we have considered are typically produced on some continuous cell function such as growth or streaming. These functions comprise separate reaction systems within the cell which are influenced by light but not controlled by it. The characteristically long latent period is thus occupied by the transmission of an effect between two relatively independent systems: the light system and the response system. Why transmission should be so much slower than in the photoreceptor which discharges a nerve impulse, we do not know. The difference obviously lies in the nature of the connecting events, but there is urgent need to know what these events really are for any cell which responds to light.

DISCUSSION

Dr. Harris: What is the explanation of phototropism in a pigmented plant which bends toward a source of light—is the action of light there also greater on the back surface?

Dr. Castle: In the case of the oat coleoptile, a much less transparent organ than the single cells we have been discussing, the action of light is greater on the side nearer the light. The effect of light is to inhibit growth on that side, due to a diminished flow of growth substance. If Bottelier's idea is correct, this effect on the transport of growth substance is based on the inhibition of protoplasmic streaming by light.

Dr. Brackett: In one case, *Phycomyces*, you postulate a direct photochemical mechanism having to do with rate of growth; in the other, *Avena*, a different mechanism affecting the distribution of growth substance. Since the spectral sensitivity curves for the two cases are so similar don't you think it more likely that we are dealing with a single, fundamental, initial photochemical action?

Dr. Castle: In all these cases which I have described having spectral sensitivity curves resembling carotinoid absorption, the initial photochemical reactions must be similar. The end-effect which we actually observe, however, is separated from the photochemical process by considerable time. This permits the occurrence of intermediate chain reactions which eventually produce the specific results which we measure.

Dr. Mestre: Has it ever been established whether the phototropic effect in *Phycomyces* is actually due to auxine? I should think this could easily be determined by growing two lots of *Phycomyces*, one in the dark and one in the light, and testing for auxine content by the usual agar block-*Avena* coleoptile method.

Dr. Castle: That test has not been made. In attempting to compare the *Phycomyces*-system with a growth system regulated by hormones, one should note that while auxine is, according to Went, strictly necessary for the elongation of *Avena* cells, the accelerator which light produces in *Phycomyces* is not necessary for growth, since this goes on adequately in darkness. At the same time, it is very interesting that Thimann and Skoog have found that the terminal buds of *Vicia faba* produce growth substance only in the light.

¹⁴ Buder, J., Ber. bot. Ges., 38, 10, 1920.

INTENSITY DISCRIMINATION

SELIG HECHT

I

Measurements of just perceptible intensity differences originally became significant because of their contribution to the problem of intensity recognition. The question which interested Fechner (1858) and his contemporaries was the relation between mind and body; what Fechner in particular wished to achieve was a quantitative description of the magnitude of sensation in terms of the physical stimulus which initiates it. Since a direct attack seemed impossible, it was replaced by an indirect one; the hope was that the estimation of sensation in terms of intensity would come out from measurements of the estimation of intensity differences.

Bouguer (1760), Steinheil (1837), Weber (1834), among others (for summary, see Hecht, 1924*b*) had shown that the sensory evaluation of intensity differences is a relative affair and depends on the prevailing intensity. None of these data before 1860 could lay claim to any high precision. In fact, it was their very approximate nature which was of significance and which made them acceptable. If I and $I + \Delta I$ are two externally measured intensities which can be recognized as just perceptibly different in sensation, then the data showed that the just perceptible increment ΔI is not an absolute value which produces its sensory effect regardless of the prevailing intensity I , but rather that the two are related so that the ratio $\Delta I/I$ is roughly constant.

The error came in when Fechner, on the basis of poor measurements, supposed the fraction $\Delta I/I$ to be rigidly constant. After adding the strategic assumption that the value of this fraction corresponds to a unit of sensation ΔS , he integrated the relations between the two, and formulated the psychophysical law that the magnitude of sensation varies as the logarithm of the stimulus intensity which evokes it. With this law, and with its superstructure of psychophysics which Fechner (1860) built we need not concern ourselves here, since insofar as they rest on the constancy of $\Delta I/I$ they have been in error for seventy-five years. Our interest is in the fraction $\Delta I/I$ itself, which by now has become of importance in its own right. In this paper we shall deal with the facts of its behavior, and with a formulation of its meaning in the field of photo-reception.

II

Fechner's supposition that $\Delta I/I$ is constant for vision had hardly appeared in print when it was shown to be erroneous by Helmholtz (1866)

on the basis of even a few measurements. However, it was Aubert in 1865 who first showed by extensive measurements with the human eye that $\Delta I/I$ is not constant, but varies in a specific way with I . His results have been corroborated by a variety of workers (see Hecht, 1924*b*, 1935*a*) during the last seventy-five years, not only for the eye but for the ear as well. For the eye $\Delta I/I$ decreases steadily from nearly 1 at low intensities to as little as 1/167 at high intensities.

The classic research on the way in which $\Delta I/I$ varies with I has been considered the work of Koenig and Brodhun (1888, 1889). They found, as had Aubert and everyone since, that as I increased $\Delta I/I$ decreased. In addition, however, they found that with further increase in intensity $\Delta I/I$ again rose. This rise at high intensities has been generally accepted, and has formed an essential part of the theoretical explanations (Hertzprung, 1905; Pütter, 1918; Hecht, 1924*b*, 1928; Houstoun, 1932) which have been given for the precise way in which $\Delta I/I$ varies with I in the visual process.

In the last two years the situation has changed fundamentally with respect to this rise in the fraction $\Delta I/I$ at high intensities. First, the intensity discrimination of insects, as measured by Wolf (1933*a, b*) with the bee, and by Hecht and Wald (1934) with *Drosophila*, shows no rise in $\Delta I/I$ at high intensities; even when tested with intensities 10,000 times higher than the one at which its minimum $\Delta I/I$ becomes established, *Drosophila* showed not the slightest upturn in the value of $\Delta I/I$. Second, the validity of the upturn for the human eye itself has also been seriously questioned. Guild (1932) published an experiment which shows that the rise in $\Delta I/I$ at high intensities is "entirely factitious and depends on the degree of adaptation of each field-intensity which prevails when the observation is made"; and quite independently, Steinhardt, in some work soon to appear from our laboratory, has shown that the rise in $\Delta I/I$ may be largely eliminated by surrounding the test-field with a large field of about the same brightness as the test-field, and by proper adaptation of the eye to the prevailing brightness.

Since the upturn in $\Delta I/I$ has been so integral a part of the theories of intensity discrimination, its non-existence in the insect eye and in the human eye has rendered a new formulation necessary for the data. I have recently (1934*b*, 1935*a*) tried to supply such a formulation, and the adequacy with which it described the data then available, as well as some data secured since

then, prompts me to present it here as the complement to a summary of the facts.

III

Intensity discrimination has been measured in insects, in *Mya*, and in man. To understand their similarities and differences, it is necessary to become familiar with the measurements. The data for *Drosophila* are in Fig. 1, and are from the

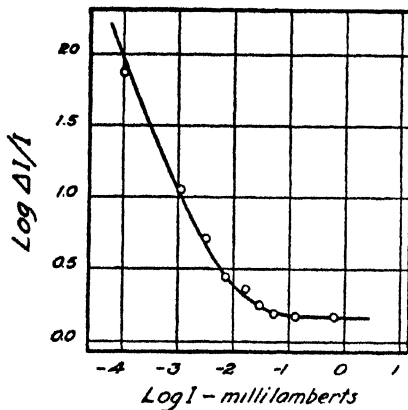


Fig. 1. The measurements of intensity discrimination of *Drosophila*. The curve is from equation (1).

work of Hecht and Wald (1934). They represent the average measurements with 24 flies, and show that $\Delta I/I$ steadily decreases as I increases. The relation between the two is continuous, such as would be expected if just one photoreceptor system were concerned. The curve drawn through the points has the form

$$\Delta I/I = c (1 + 1/KI) \quad (1)$$

where c is a constant which determines the position of the curve on the ordinates, and K is constant similarly determining the position of the curve on the abscissas. Because the data are plotted as $\log \Delta I/I$ against $\log I$, the form of the data, and of the curve resulting from equation (1) is invariant, and independent of the constants c and K . It is apparent that the equation, as represented by the curve in Fig. 1, adequately describes the data.

Wolf's (1933a) first measurements with the bee are fairly scattered and irregular. They are shown as solid circles in Fig. 2. Wolf's (1933b) later measurements, shown by clear circles in Fig. 2 are smoother, more numerous, and obviously more critical. Though they were made with different sizes of stripes, the measurements are all essentially similar. This is shown by the fact that the same curve describes them all. The curve represents the equation

$$\Delta I/I = c (1 + 1/[KI]^2) \quad (2)$$

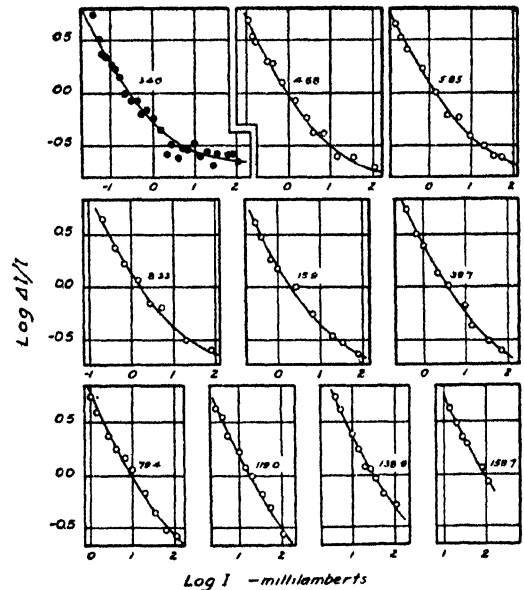


Fig. 2. Wolf's measurements of the intensity discrimination of the honey bee. The black circles are the data from the first paper; the plain circles from the second paper. The numbers attached to the curves are the visual acuities multiplied by 1000 and are inversely proportional to the size of the stripes used for the measurements. The same curve is drawn through all the data; it is from equation (2).

in which c and K have the same meaning as in equation (1); because of the log plot the shape of the data and of the curve from equation (2) is constant and, as before, independent of c and K . The curve obviously describes the later data of Wolf very well. The same curve is drawn through the earlier data, even though the measurements are not particularly critical for determining whether equation (1) or (2) fits better.

Note that equations (1) and (2) are very similar in form. Later their derivation and meaning will be discussed. For the present they may be considered as purely empirical equations which fit the data.

Drosophila and the bee have organized eyes. The only other data of intensity discrimination available for invertebrates are for the clam *Mya*, which has a diffuse sensitivity to light all over its siphon. The measurements with *Mya*, made ten years ago (Hecht, 1924a), record the necessary increase in illumination to which the animal responds with a specific reaction time, after having been adapted to another intensity. Fig. 3 shows the data for responses at five different reaction times. The measurements are not so smooth as can be wished, but they are consistent in showing that the relationship of $\Delta I/I$ to I is the same for all reaction times. Moreover the slope and shape

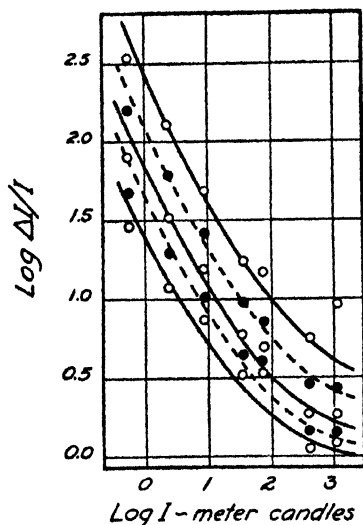


Fig. 3. Intensity discrimination of the clam, *Mya*. The clear circle so obviously off the topmost data is an extrapolated value. The curves are all from equation (2).

of the data are critical enough to show that equation (1) does not fit them at all whereas equation (2) fits them with as good precision as the data permit.

The remaining measurements of intensity discrimination are for the human eye, and furnish an unexpected demonstration of the validity of the von Kries-Parinaud duplicity theory (v. Kries, 1929) which ascribes separate functions to the cones and rods of the retina. An excellent example is in Fig. 4 which shows the measurements of Blanchard (1918) as open circles, and

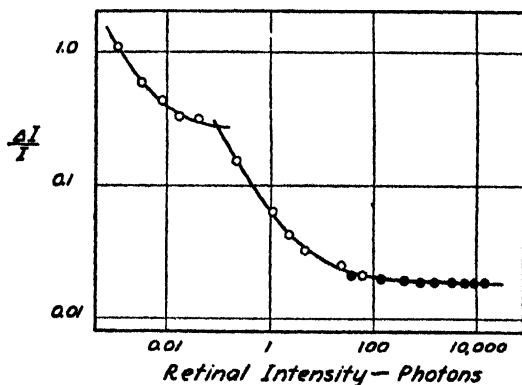


Fig. 4. The measurements of Blanchard are the plain circles; those of Lowry are the black circles and have been raised 0.15 log units along the ordinates to bring them into continuity with Blanchard's data. Note the natural breaking of the data into two sections indicative of rod and cone functions. The curve for the high intensity, cone data is from equation (2); the one for the rod data is actually from equation (1), but the other equation would do just as well.

of Lowry (1931) as solid circles, both having been made in the same laboratory under similar conditions, but thirteen years apart. The data break by themselves into two parts. Most likely the low intensity section is concerned with rod function, while the high intensity section expresses cone function.

The dichotomy of intensity discrimination into rod function and cone function may be demonstrated in two ways. The first involves the use of spectral light. The measurements in Fig. 4 were with white light. Fig. 5 contains the data

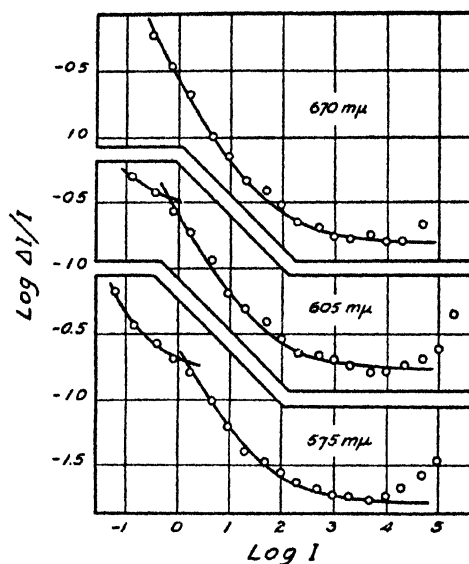


Fig. 5. The data of Koenig and Brodhun for Koenig's eye for red, orange, and yellow spectral light. The red data are continuous and show only cone function, whereas the orange and yellow show increasing amounts of rod function. The curves are from equation (1) for the rod section and from (2) for the cones.

of Koenig and Brodhun (1889) with the red, orange, and yellow light. Since the extreme red of the spectrum even at low intensities is more effective for the cones than for the rods, it is not surprising to find that the points for 670 $m\mu$ lie on one continuous curve and show no trace of the break so strikingly present with white light. The data for 605 $m\mu$ and for 575 $m\mu$ show the usual discontinuity and the separate presence of rod and cone function. As would be expected from the relative effectiveness of 605 and 575 $m\mu$ at low intensities for rods and cones (Hecht and Verrijp, 1933), the rod portion for 575 $m\mu$ is larger than for 605 $m\mu$. In all cases, the few points at high intensities must be disregarded because they were undoubtedly made under conditions which did not prevent a rise in $\Delta I/I$ as already discussed.

The second method of demonstrating the sep-

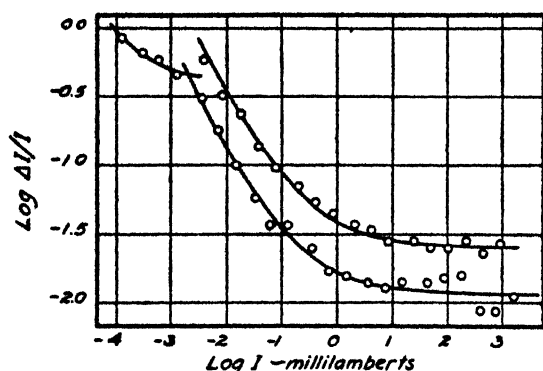


Fig. 6. Steinhardt's measurements with white light. The upper data are with a field 56' in diameter; the lower with a field 3°44' in diameter. The upper data show only cone function and are described as usual by the curve from equation (2). The lower data show both rod and cone function; the curve through the former is from equation (1), while through the latter it is from (2).

arateness of rod and cone function is by measurements with differently located retinal areas; they have been made by Steinhardt and are soon to be published. Steinhardt measured the relation of $\Delta I/I$ to I for different test-field sizes. For white light and test areas larger than 2° his measurements, without exception, fall on a double curve similar to the data of Blanchard and also of Aubert; while for smaller, foveally fixated areas, they always form single curves like those of Koenig and Brodhun with light of 670 μ . Fig. 6 shows two examples of his data. The upper measurements are for white light and a test area 56' in diameter, having a large surround in order to maintain the eye as a whole at the intensity of the measurements. This size of test-field falls entirely within the rod-free area of the fovea, and as a result the measurements show no inflection point. The lower data in Fig. 6 were made with a field 3°44' in diameter, also with a large surround, and show clearly the presence of a break indicating the presence of two separate functions, cones at high intensities and rods at low intensities.

It is striking that the curves drawn through the measurements in Figs. 4, 5, and 6 are all the same. The curves through the cone data of all observers represent equation (2). Equation (1) is definitely excluded. The precision with which the cone data are described by the curve of equation (2) is not to be underestimated, especially in view of the varied origins of the measurements. The measurements included in the rod sections are too few and cover too small an intensity range for any critical decision between equations (1) and (2). They may be described by either one; perhaps equation (1) is somewhat better, especially on the basis of Aubert's data which are

not reproduced here. The main point is that the cone data is certainly described by (2) and not by (1), while the rods may be fitted by either.

We have now examined the data of intensity discrimination with light for all the animals in which this function has been measured. The generalization which may be made about them is that from clam to insect to man they are essentially similar, and that the available measurements may be quantitatively described by one or the other of two essentially similar and very simple equations relating $\Delta I/I$ and I . We may next examine the nature and origins of these two equations.

IV

It can be shown that the two equations describing intensity discrimination may be derived in terms of certain general notions about the initial events which take place in the photoreceptor process. These general ideas are that in the process of photoreception there are (a) an inactive photosensitive substance which absorbs light and is changed by it into one or more active substances which start the train of events ending in an impulse from the receptor cell; and (b) some means of maintaining a supply of the sensitive material, since otherwise it would be used up and the process would come to an end.

Obviously, the photoreceptor process itself is more complicated than this. Moreover vision and light sensitivity involve not only the receptor process in the sensory elements but the nerve impulses generated by the stimulated elements and by neighboring elements, as well as all sorts of central nervous changes of which we know little or nothing. Since these are all concerned with vision, they surely influence its characteristics to some extent. The question is to what extent; and the answer can be secured only by trial.

Our own viewpoint has been that, no matter what determines the nature of vision, the ultimate place of origin of the impulses passing up the optic tracts is in the action of light on the sensory elements. Therefore for several years (Hecht, 1934a) we have measured various properties of vision and photoreception to ascertain whether the data owe any of their quantitative properties to the characteristics of the very first reactions which must take place between light and the sensitive elements concerned with receiving the light. The advantage of dealing with this first process is that it is photochemical, and that the properties of photochemical systems have been much studied and clearly formulated. The present data of intensity discrimination are therefore a significant case in point.

What are the properties of a photochemical system such as we have described above in gen-

eral terms, under the conditions of intensity discrimination? Let the total initial concentration of sensitive material be a ; let light of intensity I shine on it; let the concentration of photo-products be x ; and let it be assumed that some of these products reunite under proper conditions to form again the sensitive material. The velocity of the process as a whole will then be

$$(dx/dt)_I = k_1 I (a-x)^m - k_2 x^n \quad (3)$$

where m and n represent the order of the photochemical and the dark, regenerating reaction respectively; and k_1 and k_2 are their velocity constants, k_1 including the absorption coefficient. On continuous illumination a stationary state is reached in which the opposing reactions become equal; the concentrations of sensitive material and photoproducts become constant; and equation (3) becomes equal to zero. This gives

$$I = k_2 x^n / k_1 (a-x)^m. \quad (4)$$

If the system is now exposed to intensity $I + \Delta I$, the initial velocity will be

$$(dx/dt)_{I+\Delta I} = k_1 (I + \Delta I) (a-x)^m - k_2 x^n, \quad (5)$$

no changes in concentration having yet taken place. Subtracting equation (3) from (5), we get

$$(dx/dt)_{\Delta I} = k_1 \Delta I (a-x)^m. \quad (6)$$

Assume that ΔI is recognized when $(dx/dt)_{\Delta I}$ is constant and equal to c' . This probably means that in a short time Δt , a constant increment of sensitive material Δx must be decomposed by the addition of ΔI ; this small increment Δx may show itself as a given increment in the frequency of impulses leaving the receptor cell to the associated nerve fiber (cf. Adrian and Matthews, 1927). Equation (6) then gives $\Delta I = c' / k_1 (a-x)^m$. Dividing this value of ΔI by the value of I from equation (4) we get

$$\Delta I / I = c' / k_2 x^n \quad (7)$$

as a description of $\Delta I / I$ in terms of the general photoreceptor system.

In order to compare this general derivation with specific data which give $\Delta I / I$ against I , it is necessary to replace x by I derived from equation (4), and this requires specific values for m and n . When $m = n = 1$, that is when both the light and dark reactions in the initial photochemical reaction are monomolecular, equation (7) becomes

$$\Delta I / I = c (1 + 1/KI) \quad (8)$$

where $K = k_1/k_2$, and $c = c'/ak_2$. When $m = n = 2$, that is when both reactions are bimolecular, equation (7) becomes

$$\Delta I / I = c (1 + [1/KI]^{\frac{1}{2}})^2 \quad (9)$$

where $c = c'/a^2k_2$.

Equations (8) and (9) are the same as (1) and (2) which we have found to describe the data of intensity discrimination. Equation (1) describes the *Drosophila* data, and this must mean that the light and dark reactions in the photoreceptors of *Drosophila* are both monomolecular. Equation (2) fits the bee data, the data for *Mya*, and the cone data for the human eye, and indicates that for the photochemical systems in these photoreceptors, the light and dark reactions are bimolecular. For the rods of the human eye it is not possible to decide which of the two systems obtains.

The main point to be recognized is that the general quantitative properties of the very first photochemical events which take place in the visual process yield equations which adequately describe the data of intensity discrimination from a variety of widely different animals.

V

Since these ideas were published (Hecht, 1934b, 1935a), additional data have appeared which furnish fresh and unexpected confirmation of them. They are particularly interesting because their author (Wright, 1935) completely missed their significance and actually believed them to mean something quite different.

What Wright did was to pre-adapt his eye to a high intensity, and then rapidly measure the value of ΔI for a test intensity I which was a small fraction of the pre-adapting intensity. Using the same test intensity I , he measured ΔI after pre-adaptation to different high intensities. His data are shown in Fig. 7. Consider the two upper

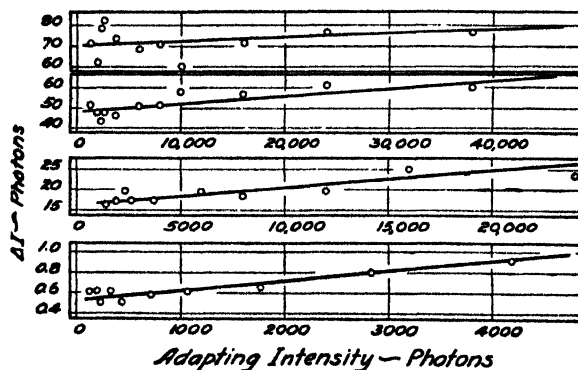


Fig. 7. Wright's measurements of ΔI for four values of I made after pre-adaptation to a varied range of high intensities. The lines indicate the general trend of the measurements.

experiments in which he used adaptation intensities between 1000 and 40,000 photons, and measured ΔI for $I = 1080$ photons and $I = 550$ photons respectively. He found that ΔI is practically constant at about 70 photons and 50 photons

respectively in spite of the large variation in adapting intensity. A slight increase in ΔI , to perhaps 15 per cent, is apparent, but this is within the accuracy of the measurements.

We have seen in equation (7) that $\Delta I/I = c'k_2/x^n$, which means that $\Delta I/I$ varies inversely with x^n . It then follows that when x varies $\Delta I/I$ will vary, and when x is constant $\Delta I/I$ is constant. Wright argued correctly that since $\Delta I/I$ depends on the concentration x , then the value of $\Delta I/I$ should vary with the state of adaptation of the eye, which can be controlled by the adapting intensity. However, it is significant to note in Fig. 4 that $\Delta I/I$ becomes practically constant, and therefore x becomes constant, at about 100 photons. Between 1000 and 40,000 photons, the state of adaptation of the eye as judged by $\Delta I/I$, and of the photoreceptor system as judged by x , is constant. In other words, though Wright thought he had changed profoundly the state of adaptation of the eye with these high intensities, the fact is that it had remained practically unaltered. This is confirmed by the fact that not only $\Delta I/I$, but visual acuity (Koenig, 1897), and the critical fusion frequency for intermittent stimulation (unpublished experiments) are practically constant in the range of these high illuminations.

Let I_a be any one of these very high intensities and let x_a be the concentration of photoproducts at the stationary state when the eye is adapted to I_a . The concentration of sensitive material is then $a-x_a$. In Wright's experiment, the eye was rapidly changed from this intensity I_a to a photometric field of which the intensity on one side is I and on the other the just noticeably brighter one $I+\Delta I$. The action of I and of $I+\Delta I$ on the system is then given by equations (3) and (5) in which $a-x_a$ is the concentration of sensitive material and corresponds to the pre-adapting intensity I_a . Following the steps already given and remembering that $m = n = 2$ for the cones, we get that

$$\Delta I = c/(a-x_a)^2 \quad (10)$$

where $c = c'/k_1$.

Fig. 4 tells us that x_a is practically constant between 1000 and 40,000 photons; hence $a-x_a$ is constant. In terms of equation (10), ΔI should be practically constant, and this is what Wright found. The values of ΔI in the two upper experiments in Fig. 7 rise only slightly as I_a mounts to 40,000 photons, as they should since x_a also increases only very slightly.

Returning to Fig. 4, we see that as I goes below 1000 photons, $\Delta I/I$ begins to rise, at first slowly and then rapidly. In terms of equation (7) this means that x_a decreases in the same way, and that therefore $a-x_a$ increases similarly. There-

fore for adaptation intensities in this range, ΔI should not be constant, but should increase with adapting intensity. The lowest block of data in Fig. 7 describes just such an experiment made by Wright. I_a varies between 100 and 4,000 photons, while I is 6 photons. The data clearly show that over the range covered, ΔI increases about 60 per cent. The third block of measurements in Fig. 7 were made by Wright with adaptation intensities intermediate between the lower and higher just discussed, and also show an intermediate rise in ΔI . These experiments thus yield results which are to be expected in terms of the ideas about intensity discrimination which have been presented here.

Wright also made some measurements which are the complement of those already discussed. In these he kept the adapting intensity I_a constant, and varied the measuring intensity I , and then determined the value of ΔI corresponding to it. In the one experiment given in his paper $I_a = 10,000$ photons while I varies between 50 and 550 photons.

What can we expect of the behavior of $\Delta I/I$ under these conditions? Equation (7) tells us that $\Delta I/I$ is inversely proportional to the concentration of photoproducts at the stationary state. In the present case the adapting light I_a keeps x constant at x_a ; therefore $\Delta I/I$ must be constant. This is precisely what Wright found; and here again his data do precisely what is to be expected of them.

Perhaps the simplest way of realizing what Wright's experiments mean in terms of the photoreceptor process is to look again at the data in Fig. 4. Between 100 and 10,000 photons the eye is in a practically constant state as shown by the constancy of $\Delta I/I$. It matters little how high the adapting intensity is, and whether the measuring intensity is 100 or 1000 photons, because the concentration x is nearly the same for this range, and therefore $\Delta I/I$ will be the same. Wright's experiments probably also involve a certain amount of dark adaptation, but it would take us too far afield to go into the matter here (cf. Hecht, 1935b).

For us the significance of Wright's measurements is that they are easily interpreted by, and necessarily follow from the equations and ideas already used for describing intensity discrimination in terms of the photochemical changes in the retinal elements during vision. They may thus be considered as added and unprejudiced confirmation of these ideas.

SUMMARY

An examination of the data of intensity discrimination of such diverse animals as the bee, the fruit-fly, the clam, and man, shows them to

be essentially similar. In general the fraction $\Delta I/I$ decreases as I increases. The measurements differ in the fact that for man the data break into two sections indicative of rod and of cone behavior, whereas for the other animals the behavior of only one sensory system is apparent.

The data for each photosensory system is described quantitatively by one of two essentially similar equations relating $\Delta I/I$ to I .

A theory of visual intensity discrimination is proposed in terms of the photochemical events which take place at the moment when a photosensory system already adapted to the intensity I is exposed to the just perceptibly high intensity $I + \Delta I$. Unlike previous formulations this theory predicts that the fraction $\Delta I/I$, after rapidly decreasing as I increases, does *not* increase again at high intensities, but reaches a constant value which is maintained even at the highest intensities. This theory yields the two equations used to describe the measurements.

In terms of the theory, the data of intensity discrimination give critical information about the order of both the photochemical and dark reactions in each photosensory system. The reactions turn out to be variously monomolecular and bimolecular for the different animals.

Recently published experiments show that when the eye is pre-adapted to very high intensities, the instantaneous value of ΔI for a given I is very nearly constant when adapting and measuring intensities are both high, but increases significantly when they are both lower. Moreover when the pre-adaptation intensity is high and constant and the measuring intensity I is variable, then $\Delta I/I$ remains constant.

The results of these measurements are shown to follow from the equations and ideas already formulated, and thus constitute independent corroboration of the theory.

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INTERMITTENT LIGHT STIMULATION AND THE DUPLICITY THEORY OF VISION

SELIG HECHT, SIMON SCHLAER, EMIL L. SMITH

I.

The most consistently revealing generalization about vision comes from Max Schultze's (1866) recognition that the vertebrate retina in general and the human retina in particular contain two different types of receptor, rods and cones, and that these have different visual functions. Further physiological implications of this double retinal structure were drawn by Parinaud (1885) and by von Kries (for summary see v. Kries, 1929), and the total conception has formed the Duplicity Theory which ascribes the characteristics of colorless vision at low intensities to the rods, and of color vision at high intensities to the cones.

With the years the evidence for this generalization has become more extensive and impressive because of the variety of visual measurements which have found their simple explanation in terms of it. We wish to add to this body of data the information gained from the work on flicker which has been going on in our laboratory for the last five years.

II.

The flickering sensation produced by regularly interrupted illumination disappears when the frequency of interruptions is made sufficiently high. Under controlled conditions the determination of this critical fusion frequency may be made with considerable accuracy. As a result, its precise value has been shown to depend on a variety of conditions.

The most basic factor which controls the critical frequency is the intensity of the flickering light. Though the dependence of the critical frequency on illumination was recognized over one hundred years ago by Plateau (1829), and is evident from the work of Emsmann (1854) and of Nichols (1884), it was only forty years ago that Ferry (1892) formulated what has since become known as the Ferry-Porter law, namely, that the critical frequency is proportional to the logarithm of the illumination intensity. Ferry's published measurements distinctly do not support this generalization, but the later data of Porter (1902) do. Porter's work was corroborated by Kennelly and Whiting (1907), by Ives (1912), and by Luckiesh (1914).

Porter found that when the critical fusion frequency—as cycles of light and dark per second—is plotted against the logarithm of the intensity the data fall on *two* straight lines instead of one. The two lines intersect at an

illumination of about 0.25 meter candles, and the slope of the lower is 1.56 while that of the upper is 12.4. These findings were confirmed by Ives. Ives data for different parts of the spectrum show a dual logarithmic relation similar to that for white light. However, the slope of the straight lines and their point of intersection seem to vary with the wavelength of the light, the upper and lower limbs of the relationship varying in different ways. In addition Ives found the extraordinary fact that for blue light the lower line becomes horizontal.

These peculiarities are difficult to reconcile with the obvious interpretation of Porter's data in terms of the Duplicity Theory, that is, that the lower limb describes the function of the rods while the upper limb describes the function of the cones. This difficulty has been emphasized by Allen (1919, 1926) who has in general agreed with the work of Porter and of Ives, but has differed from them by drawing through his measurements about five short straight lines of different slope instead of the usual two. In our estimation, the data presented by Allen do not justify this treatment; the points appear to lie on a continuously curving line. The work of Lythgoe and Tansley (1929), distinctly gives no support to Allen's multiplicity of straight lines.

Lythgoe and Tansley's measurements confirm the logarithmic relation of intensity to fusion frequency, but Lythgoe and Tansley attach no importance to its strict formulation as done by Ferry, by Porter, and by Ives, and consider that their data agree only under certain conditions with the linear relation of critical frequency to $\log I$. The same may be said about the measurements of Granit and Harper (1930), who found that for a range of about 1 to 1000 in intensity the critical frequency is very nearly directly proportional to the logarithm of the intensity. For higher intensities the relationship does not hold, and the curve of frequency against $\log I$ tends to become horizontal, as already found by Grünbaum (1898).

One striking thing appears in the work of Lythgoe and Tansley though they do not recognize its significance. Ives had found that for blue light the lower limb of his data is horizontal, and in this he had been confirmed by Allen. This seemed a special property of blue light. However, Lythgoe and Tansley have recorded that when measurements are made with a retinal area 10° from the center of the eye the lower portion of the data tends to be horizontal even for white light.

Our original determination (Hecht and Verrijp, 1933*b*) to study flicker was prompted first, by the confused state of the situation in its relation to rod and cone functions; and second, by the fact that none of the measurements existing at the time covered a range of intensities sufficient to define the relation between critical frequency and intensity over the functional range of the eye. Since then, we have measured this relation for different portions of the retina, for different sizes of field and for different colors, for as large a range of illuminations as possible, and under such conditions of fixation and surrounding illumination as to render the data reproducible and definitive.

III.

For a central area approximately 2° in diameter the retina is practically rod-free and contains only cones. Outside of this area, rods appear and increase in number toward the periphery. Judging by this fact, and by previous work on intensity discrimination (Steinhardt, in press; cf. the preceding paper in this Symposium), and on dark adaptation (Hecht, Wald, and Haig, 1932), the relation between fusion frequency and intensity as measured with central areas smaller than 2° in diameter should be a continuous function representing cones, whereas with larger areas or with similar small areas outside the fovea, the relation should show a duplex character illustrative of the predominant working of rods at low intensities, and of cones at high intensities.

The measurements of Hecht and Verrijp (1933*b*) with a small field located centrally and peripherally on the retina showed this to be correct, and our more recent measurements (Hecht and Smith, 1935) with centrally fixated areas of varying size add further confirmation to these findings.

The apparatus originally used (Hecht, Schlaer, and Verrijp, 1933) was so arranged that an observer, looking through a pupil of fixed dimensions, viewed a field of 2° diameter whose illumination was periodically interrupted and which was surrounded by a field of 10° whose illumination was continuous but otherwise identical with the interrupted field. We have since redesigned this optical system so as to increase the size of the surround to 35° in diameter, and to enable us to have flickering test-fields of any size within the limits set by the diameter of the surround. The rest of the apparatus is the same as before, and various parts of it are concerned with controlling and recording the retinal position of the field, its intensity, its spectral composition, the frequency of interruption of its illumination, and the location and brightness of a fixation

point in it. The procedure for making measurements is so simplified and regulated that a complete set of readings over the whole intensity range of vision can be made at one sitting without fatigue or strain.

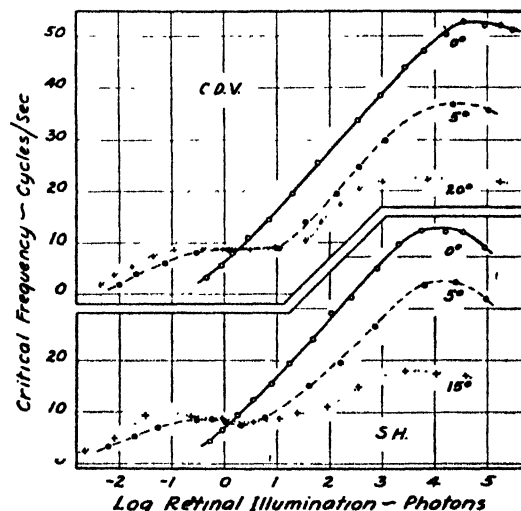


Fig. 1. Relation between critical frequency and $\log I$ for white light with a 2° field in four retinal locations: at the fovea, and at 5° , 15° and 20° above the fovea. The data are from Hecht and Verrijp (1933*b*). Due to an error in the original paper, the intensities have had to be multiplied by 40 to convert them correctly into those here given.

Fig. 1 shows the measurements of Hecht and Verrijp (1933*b*) made with a 2° field (having a 10° surround) situated in the center of the fovea and at 5° , 15° , and 20° above it. It is clear that for the fovea there is one continuous relation between critical frequency and the logarithm of the intensity. The relationship, however, is not rectilinear, but distinctly sigmoid, the S-shape being rather drawn out. In the range of intensities lying between about 10 and 1000 photons, the data lie with reasonable precision on a straight line, and thus confirm Porter, Ives and the other workers. The slope of this middle section is 11.0 and is the same magnitude as found by previous investigators.

Below 10 photons the critical frequency continues to decrease as $\log I$ decreases, forming a gentle curve and stopping fairly abruptly when with central fixation the field appears uniform even when the test area is extinguished. At the highest intensities the relation between critical frequency and $\log I$ rapidly ceases to be linear. As the intensity is raised a maximum critical frequency is soon reached, beyond which a further increase in intensity results in no further increase in critical frequency; rather it results in a decrease.

The measurements with the peripheral fields shown in Fig. 1 clearly fall into two parts. The first is at low intensities, where the critical frequency first rises with $\log I$ and then reaches a maximum which is maintained approximately constant for about 1.5 logarithmic units. The total intensity range covered by this rise and plateau is about 3.5 logarithmic units. The second part also begins with a rise in critical frequency as $\log I$ increases, and also terminates when the critical frequency reaches a maximum. The intensity range covered by the second part is about 4 logarithmic units. The same results obtain in whatever peripheral direction of the eye the measurements are made.

Since the central, 2° field falls within the rod-free area of the retina, the continuous nature of the data indicates that they are a function of the cones alone. The double nature of the peripheral measurements very likely represent rod function for the low intensity section and cone function for the high intensity section. This is borne out by the increasing separation of the two sections as measurements are made farther and farther from the center: the cone section shifts to higher intensities and the rod section to lower intensities, as would be expected from the increasing ratio of rods to cones in these regions.

IV.

We have very recently measured the relation between critical fusion frequency and intensity for four centrally located areas 0.3° , 2° , 6° , and 19° in diameter, and our measurements (Hecht and Smith, 1935) confirm these conclusions. The surround for all the test fields in these new measurements has the same diameter, 35° . This increase in size of surround was for the purpose of removing, if possible, the drop in critical frequency which occurs at very high intensities after

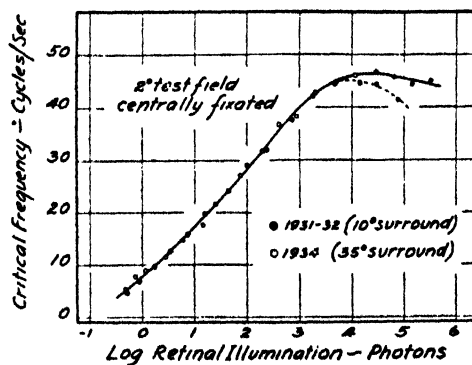


Fig. 2. Comparison of previous measurements (Hecht and Verrijp, 1933b) using a 2° field having a 10° surround with those recently made with the same eye and field but having a 30° surround.

the critical frequency has reached a maximum. Fig. 2 shows the data of S. H. with the 2° test field, using a surround of 35° and of 10° . The two sets of data are practically identical except at high intensities where the large surround observations show only a slight decrease in critical frequency beyond the maximum. Even this decrease is frequently absent; again and again we have made runs in which the top of the curve is entirely flat.

The measurements with these four central fields for E. L. S. are shown in Fig. 3. As expected, the data for 6° and 19° break into two sections which must be identified with rod function at low intensities and with cone function at

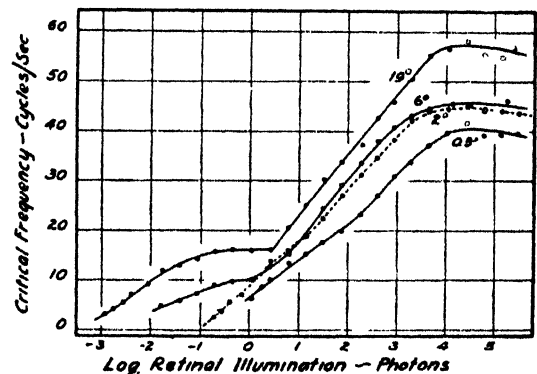


Fig. 3. Influence of the area of test field on the relation between critical frequency and $\log I$.

high intensities. Note that the rod part is less extensive, and its plateau lower for the 6° field than for the 19° field. For the 2° and 0.3° fields the rod part is definitely missing. With the slight bend in these data we need not now concern ourselves except to say that we are certain it is not due to a slight admixture of rods.

The data for the 6° and 2° fields are of pointed interest in the problem of flicker and area. Except for the absence of the rod piece in the smaller field, the two sets of data are almost identical. Under the circumstances of possessing the same surround, a ninefold increase in area of the test field hardly changes the relation of critical frequency to intensity so far as cone function is concerned (cf. Granit and Harper, 1930).

Fig. 4 presents the data of S. H. as the logarithm of the critical frequency (f) against the logarithm of the intensity (I). This type of plot shows more strikingly the phenomena already described. In spite of the irregularity in the 0.3° data, a single curve describes the measurements fairly well. The same single curve is even more expressive of the 2° data, and it is also

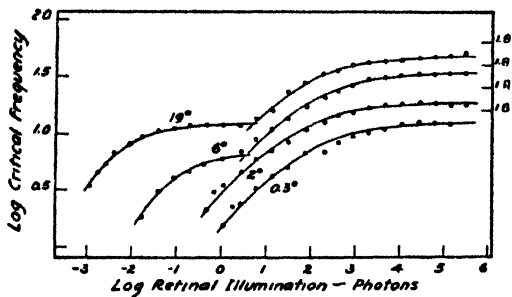


Fig. 4. Area and the flicker relation. The log I axis is the same for all the data. The numbers on the log frequency axis to the left apply to the uppermost data only; the other data have been moved down in steps of 0.2 log units in order to space them, and their precise position is given on the right. The curves are from equation (1) for the cone portions and from (2) for the rod portions.

drawn through the cone portions of the 19° and 6° data. Its equation is

$$KI = f^2 / (f_{max} - f)^2 \quad (1)$$

where K is the dimensional constant which determines the position of the curve on the intensity axis, just as the value of f_{max} determines its position on the critical frequency axis. Neither K nor f_{max} affect the shape of the curve on the log I -log f plot.

The rod sections of 19° and 6° require a slightly different curve which is the same for the two fields. Its equation is

$$KI = f / (f_{max} - f)^2 \quad (2)$$

where the terms have the same meaning as before.

It is worth emphasizing that the rod sections of the 19° and 6° fields have the same curve drawn through them. While this is not clearly seen in an ordinary plot of critical fusion frequency against log I , it becomes plain in the log f -log I plot of Fig. 4. The identity of the curves shows that the difference between the 19° and 6° rod data is not basic, but merely represents a displacement on the axes in the log plot corresponding to a change in the scale of plotting on the ordinary plot. Exactly the same is true for any systematic differences which the cone data show. Fundamentally the systems in the rods and cones which determine the relation between critical frequency and intensity remain the same regardless of area. Only the dimensional constants are changed by changing the area.

V.

In order to confirm the identification of the two sections of all these measurements with rod and cone function, we have used different parts of the spectrum to study the relation of critical fre-

quency to intensity. Fig. 5 gives the relative spectral sensitivities of the cones and rods, and shows what may be expected of the measurements. Spectral energy can produce no visual effect until it reaches the relative intensity indi-

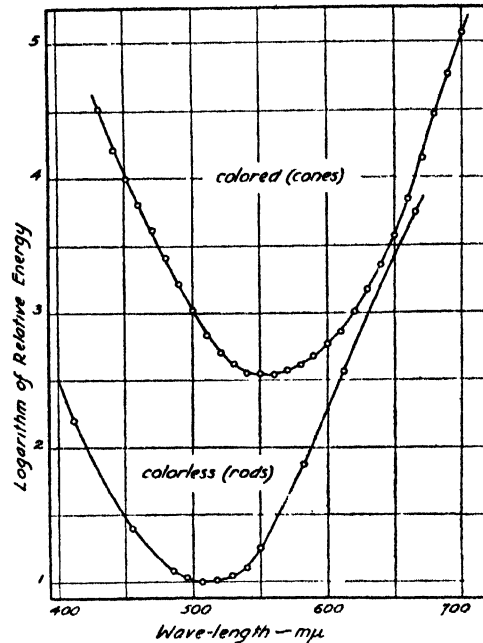


Fig. 5. Comparison of rod and cone sensibility distributions in the spectrum, taken from the data of Hyde, Forsythe and Cady (1918) and of Hecht and Williams (1922). The curves are each accurately drawn from their separate data. Their vertical separation, however, has been arbitrarily arranged so that they are nearly coincident in the red; this is a graphic expression of the fact that the colorless and color thresholds of the eye are nearly the same in the red.

cated by the rod curve. Above that, rod function dominates until the cone threshold is reached. The intensity distance over which the rods dominate in visual function changes throughout the spectrum: between 670 and 630 $m\mu$ it is small and alters only slowly; beginning at about 600 $m\mu$ and going toward the blue, the distance becomes rapidly larger, while below 500 $m\mu$ it remains practically constant.

Preliminary investigation (Hecht and Verrijp, 1933a) showed these expectations to be correct. We have therefore measured in detail (Hecht and Schlaer, 1935) the relation between intensity and critical frequency for different parts of the spectrum with a circular test field 19° in diameter, surrounded by a non-flickering area 35° in diameter.

The information conveyed by the measurements can best be understood from their graphic

representation. As Fig. 6 shows, the data break sharply into two sections. The high intensity portions, identified with cone function, fall together for all the colors. The low intensity sections, identified with rod function, are spread out much as expected, and extend to lower and lower intensities with decreasing wavelength.

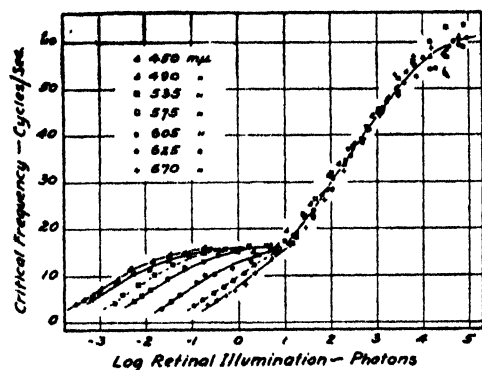


Fig. 6. The data of S. H. showing the relation of critical frequency to $\log I$ for the different spectral regions indicated.

Fig. 6 resolves the mystery of Ives' old measurements showing that the low intensity portions of critical frequency data which he found for different parts of the spectrum may be represented by straight lines which differ in slope, the red being steepest and the violet being practically horizontal. It is apparent in Fig. 6 that for short stretches near the rod-cone transition, straight lines can be drawn through the rod data, showing different slopes for the different wavelengths.

The real phenomenon, however, is something quite different. It is that the separation of rod

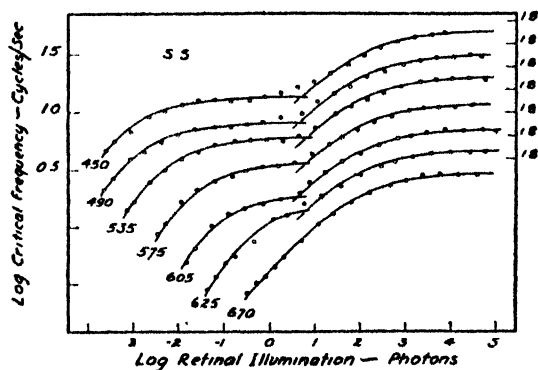


Fig. 7. The data of S. S. plotted as log frequency against $\log I$ for the different spectral regions. The numbers on the ordinates to the left apply to the topmost data; for convenience, the others have been moved down in steps of 0.2 log units, and their exact positions are indicated to the right. The curves are from equation (1) for the cone portions and from equation (2) for the rod portions.

and cone sections as a whole increases as the wavelength decreases. This is shown strikingly by Fig. 7 in which the data of S. S. are plotted as the logarithm of the critical frequency against the logarithm of the intensity. The data for 670 $m\mu$ fall on a single, continuous curve, whereas the data for all other parts of the spectrum are best described by two separate curves. The transition between the two portions is quite sharp for all but the blue and violet data. The high-intensity, cone curve is in the same position for all colors, and the only effect of changing the spectral composition of the light is to shift the position of the low-intensity, rod curve along the intensity axis, without in the least changing its form.

The curve which in Fig. 7 is drawn through the data for 670 $m\mu$ represents equation (1) previously used for the cones in Fig. 4. It is apparent that the equation describes the entire 670 $m\mu$ data with precision. The same curve has been drawn through the cone portions of all the other parts of the spectrum, even though it slightly the transition points for the blue and violet. The rod portion of all the measurements has the curve drawn through it from equation (2) also as in Fig. 4.

The identification of rod and cone function is borne out by subjective observation. At low intensities and below the critical fusion frequency the flicker is distinctly located in the peripheral portion of this 19° field so that the field resembles a flickering doughnut, and the last appearance of flicker is always in the periphery. With increasing intensity, the first sign of approaching cone function is the appearance of color in the field, which becomes identifiable with certainty about 0.5 log unit below the actual inflection point of the measurements. At the intensities around the transition, two separate loci of flicker are very often apparent near the critical frequency: one in the periphery, and the other in the center. At intensities higher than the transition intensity but near it, flicker usually persists longest in the center, but beyond these intensities the last trace of flicker may be in any part of the field. Obviously the rods determine the low intensity section, and the cones the high intensity section, but the specific cones which set the critical frequency are not necessarily the same throughout the high intensity section.

VI.

The main purpose of this paper has been to show that the phenomena of flicker furnish a new and substantial support for the Duplicity Theory. It is hardly necessary to labor this point. We propose instead in this closing section to discuss

the relation of the quantitative aspects of the data to the photoreceptor process previously used for describing vision (Hecht, 1934) and already presented in general form in the preceding paper on Intensity Discrimination given at this Symposium.

The data are so new, and before their appearance were so generally unexpected, that as Hecht and Verrijp (1933c) have shown none of the older formulations (e.g. Fick, 1863; S. Exner, 1870; K. Exner, 1870; Troland, 1913; Ives, 1922; Lasareff, 1926) contribute significantly to an understanding of them. In terms of our newer knowledge Hecht and Wolf (1932) have derived Talbot's law from first principles; and Arnold and Winsor (1934) have shown that Talbot's law must involve the entrance of the light intensity factor as a first power in the equations. Hecht and Verrijp (1933c) have already defined the fundamentals for the theoretical description of flicker; we shall rely on this derivation and give it in a simpler and more general form so that its implication for the present data will be easily apparent.

Assuming the first step in the photoreceptor process to be a reversible photochemical reaction in which a sensitive substance is changed by the light into photoproducts which recombine under certain conditions to form the original substance, then the velocity of the process as a whole under the influence of light is

$$(dx/dt)_{\text{light}} = k_1 I (a - x)^m - k_2 x^n \quad (3)$$

where I is the intensity, a the initial concentration of sensitive material, x the concentration of photoproducts, and m and n represent the order of the photochemical and of the dark, regenerating reactions respectively. In the absence of light, only the "dark", regenerating reaction goes, and the equation

$$-(dx/dt)_{\text{dark}} = k_2 x^n \quad (4)$$

gives the rate at which it forms the photosensitive material.

In intermittent illumination these two reactions alternate rapidly, and at the disappearance of flicker, they form a steady state in which what has been decomposed during the light period is regenerated during the dark period. Since the light and dark periods in all our measurements are equal, the light and "dark" velocities will be equal. Putting equation (3) equal to (4) and solving, gives

$$KI = x^n / (a - x)^m \quad (5)$$

where $K = 2k_1/k_2$. It is at once apparent that equations (1) and (2) whose curves have been drawn through the flicker data in Fig. 4 and in

Fig. 7 are this steady state equation (5) in which the critical frequency f has been made proportional to the concentration x , and m and n have been given specific values of 1 or 2.

Four varieties of equation (5) are shown in Fig. 8. The value of n determines the slope of the steep limb of the curves, whereas that of m controls the curvature of the bend joining the steep limb with the horizontal one.

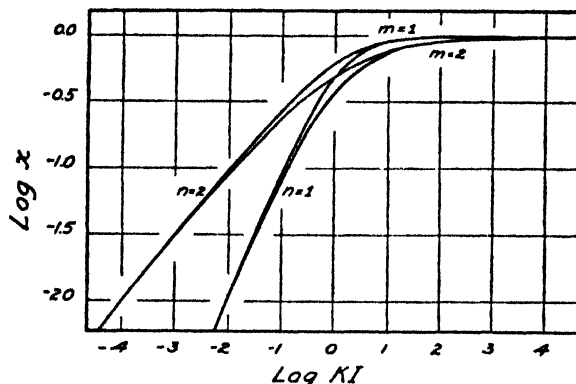


Fig. 8. The steady state equation (5) plotted when m and n are each 1 and 2. Because of the log plot the shape of the curves remains constant regardless of the values of K and a .

Examination of the data in Fig. 4 and in Fig. 7 shows that the rod curve always has twice the slope of the cone curve. This determines the value of n in the two cases; $n = 1$ for the rods, and $n = 2$ for the cones. The best curve to fit the cone data always has $n = 2$, and $m = 2$, as was found also for intensity discrimination (Hecht, see the preceding paper). The rods, however, are somewhat variable with regard to the value of m . This is illustrated by Fig. 9

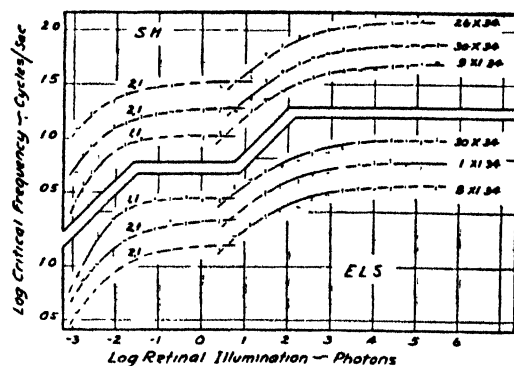


Fig. 9. The single, individual measurements as taken in the course of a run. For convenience, the separate runs (dated to the right) have been spaced 0.2 log units apart on the vertical axis; the values on the ordinate scale refer only to the topmost run for each investigator. The numbers attached to the rod curves indicate the values of m and n in equation (5) used in drawing them.

which contains the individual measurements of two of us with the 19° field and with white light. Besides showing the adequacy and reproducibility of the data, especially in relation to the curves, Fig. 9 indicates this systematic variability of the rod measurements. Of the six runs, the rod data of four are described adequately by equation (5) only when $m = 2$, while the two others are better fitted by $m = 1$. The rod measurements of Hecht and Verrijp (1933b) are also better fitted, by far, when $m = 1$. Fig. 8 shows that when $m = 2$, $n = 1$, the curvature is more gradual, whereas when $m = 1$, $n = 1$, the transition between the rising limb and the plateau is sharper. Also, the plateau itself continues to rise gently in the 2,1 curve, whereas it flattens off quite rapidly in the 1,1 curve.

Whether these differences really represent daily variations in the state of the rod photoreceptor system, it is hard to say. The consistency with which either one or the other type of curve appears is, however, impressive for us who have watched them for many months.

The assumption that the critical frequency f is proportional to the steady state concentration x means very simply for the rods that f is determined by the velocity of the dark reaction as given by equation (4) since for the rods $n = 1$ always. For the cones, however, this assumption, at first sight, produces a paradoxical situation. The data clearly show that for the cones $n = 2$, so that if f is determined by the "dark" velocity it should be proportional to x^2 . Yet the data follow equation (1) only when f is made proportional to x .

A simple and realistic way of resolving this contradiction is to suppose that the proportionality of f to x indicates the dependence of the critical frequency for the cones not on the dark reaction which re-forms the sensitive material, but on the secondary dark reaction which follows the photochemical one in time and which uses the photoproducts to form impulses that leave the cell to the nerve. There is no reason to suppose that the velocity of this reaction is anything but directly proportional to the concentration of specifically useful photoproduct rather than to its square.

SUMMARY

1. In the retina, central areas whose diameter is less than 2° possess only cones, while larger areas or those more peripherally situated, have rods and cones. In conformity with this, the relation of critical fusion frequency to intensity is a single function for centrally fixated areas below 2° , and a double function for similarly fixated, larger areas, and for small areas peripherally fixated. The two sections of such data

are easily identified with rod activity at low intensities and with cone activity at high intensities.

2. The equations describing the rod data are the same for all areas whatever their situation, differing only in the values of the associated dimensional constants which control the location of the curves on the coordinate axes. Similarly the equations for the cone data are the same for all areas and locations; and change of maximal frequency with area or location is the expression merely of the value of a constant which determines the position of the data on the frequency axis. Area and location therefore do not influence the fundamental nature of the flicker relation through each receptor system, but merely alter the extraneous constants of the relation.

3. Judging by the relative spectral sensibilities of rods and cones, the positions of the flicker functions for rods and cones on the intensity scale should separate increasingly as the wavelength decreases. Measurements with a centrally located test field of 19° diameter confirm this by showing that the total intensity range of the combined flicker functions is smallest in the red, and increases steadily toward the blue. The increase is due entirely to the extent of the low intensity, rod section which is practically nonexistent in the red and largest in the violet. The high intensity cone portion for all colors is in the same position on the intensity axis, and the only effect of decreasing wavelength is to shift the rod section to lower intensities without changing its shape.

4. The measurements are faithfully described by two similar equations, one for the rods and one for the cones, both equations being derived from the general stationary state equation already used for various visual functions.

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THE DISCHARGE OF NERVE IMPULSES FROM THE SINGLE VISUAL SENSE CELL

H. KEFFER HARTLINE

One of the most important chemical reactions in living systems is that initiated in photoreceptive organs by light, for this photochemical activity ultimately gives rise to the nerve messages which keep the organism informed concerning one of the most significant elements of its environment. These messages, consisting of impulses in afferent nerve fibers, originate in the visual sense cells, each one of which signals the condition of illumination upon it. Upon their integration depend all the phenomena of vision. It is the purpose of this paper to describe some of the features of the activity in the nerve fiber from the visual sense cell, and to discuss briefly the peripheral mechanism whereby light energy gives rise to this activity.

The development of methods for intercepting the afferent messages from single sensory elements has yielded a powerful tool for the study of the physiology of the sense organs. These methods depend upon the amplification and oscillographic recording of action potentials in the sensory nerve fibers, combined with procedures for limiting the activity to a single fiber. This may be accomplished either by cutting all but one of the fibers in a sensory nerve between the stimulated region and the recording electrodes (Adrian and Bronk (3); Bronk (8)), or by stimulating only one receptor (Adrian and Zotterman (6); Adrian and Unrath (5); Matthews (21)). Frequently these two procedures may be combined.

Thus it is possible to study the response of the individual sensory ending, in terms of the impulses propagated in its attached nerve fiber. Such studies have yielded information invaluable both in the interpretation of sensory mechanisms and in the discussion of the central effects of sense organ activity (Adrian (1), (2); Bronk (9)).

Application of these methods to the single photoreceptor cell necessitates first of all a suitable preparation, from which the true, unmodified sensory discharge may be recorded. Associated with the actual photosensitive cells in the eyes of most of the higher animals are nervous elements such as ganglion cells and synapses; it is important to avoid the complications which these elements may introduce. The lateral eye of *Limulus polyphemus* provides a preparation which does this. In this eye the optic nerve fibers come directly from the light sensitive retinula cells, and it is possible to record the impulses in a single fiber in response to illumination of the corresponding retinula cell (Hartline and Graham (16)). Records from such a preparation are shown in Figure 1. The discharge of impulses begins, after a short latent period, with an initial burst at relatively high frequency; as the sense cell becomes adapted to the light, the frequency of impulses subsides to a steady level which is maintained as long as the light continues to shine. This figure also shows the effect of intensity of

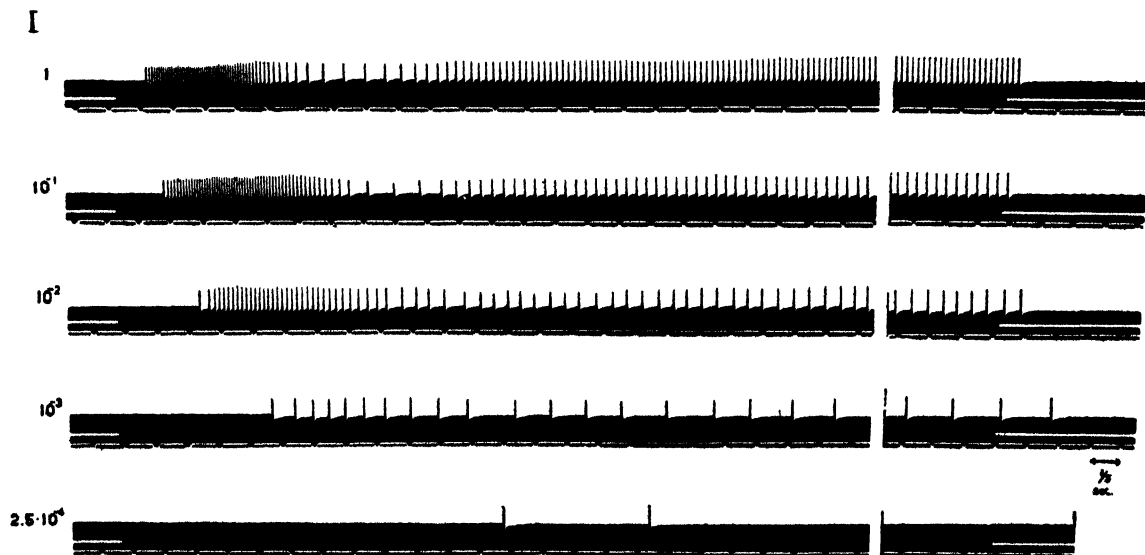


Figure 1. Oscillographic records of the action potentials in a single fiber from the eye of *Limulus*, in response to illumination of its attached sense cell by lights of various intensities. Relative intensities are given at the left of each record. The time marker beats 1/5 seconds; above its record is the signal marking the period of illumination.

stimulating light upon the impulse discharge. The higher the intensity, the shorter is the latent period and the higher the frequency, both in the initial burst and final steady level—in short, the entire response is accelerated.

The responses described above constitute the typical afferent nerve message from the photoreceptor cell. In all their broader features they are also typical of sense organ activity in general, particularly of the more slowly adapting sensory endings such as pressure and muscle tension receptors (Bronk and Stella (10); Matthews (21)). This striking uniformity in the behavior of sensory end organs of widely different types is an indication that the actual excitation of the terminal portion of the nerve fiber is accomplished in much the same way in all of them and is probably dependent on general properties of nervous tissue (Adrian (1)). The excitation of the end organ itself, on the other hand, necessitates a highly specialized mechanism, different for each type of adequate stimulus.

In the case of the visual sense cell, this special mechanism is a photochemical system, whose properties have been studied in detail. Hecht (18) has successfully related many of the fundamental phenomena of vision to this photochemical basis of photoreception. Indeed, of all the processes, from the initial action of light on the receptor cell to the final production of nerve impulses, this is the only one about which there is any considerable amount of knowledge.

It is profitable to extend some of these studies to the single visual sense cell. As an example we may consider the effect of wavelength of stimulating light (Graham and Hartline (13)). Light of given energy content, but from various parts of the spectrum, does affect the visual sense cell from the *Limulus* eye differently, depending upon the wavelength, but the differences are due entirely to brightness inequality. If the intensities of lights of different wavelengths are adjusted to suitable values, the responses will be identical, impulse for impulse. The single visual sense cell, therefore, cannot distinguish wavelength, but can only gauge the brightness of the stimulating light. The reciprocals of the intensities necessary to produce a given constant response are by definition the visibilities at the respective wavelengths; these visibility values form a simple curve (Figure 2), symmetrical about a maximum at 510-520 $m\mu$ and closely similar to the visibility curve of the human eye for dim vision. Since the amount of photochemical change in any system depends upon the absorbed intensity, and since constancy of response indicates a constant amount of photochemical change in the sense cell, we may follow Hecht and Williams (19) in inter-

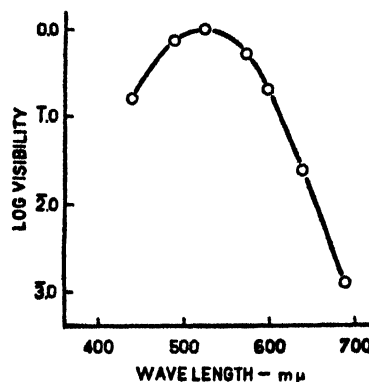


Figure 2. Visibility curve of a single visual sense cell from the eye of *Limulus*. (cf. Graham and Hartline (13)).

preting the visibility curve as simply the absorption spectrum of the photosensitive substance primarily responsible for the excitation of the sense cell.

Another example of the photochemical nature of the excitation of the visual sense cell is to be seen in the relative roles of intensity and duration (Hartline (15)). With short flashes of light, increased duration of flash decreases the latent period of the response, increases the frequency of impulse discharge, and produces a greater total number of impulses. In short, increased duration affects the response in precisely the same manner as does increased intensity. Indeed, the reciprocity between intensity and duration is complete, at least for short flashes. This may be seen in Figure 3, in which the

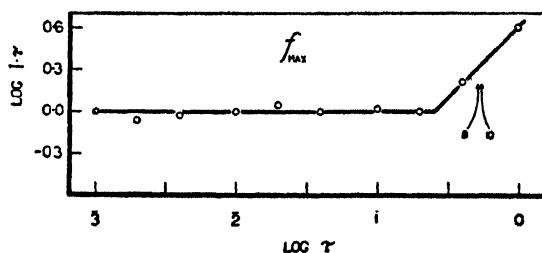


Figure 3. Energy of flash necessary to produce a constant response (maximum frequency of 56 impulses per second), plotted as a function of duration of flash (seconds). Unit of energy = 3×10^3 meter candle seconds. Double logarithmic plotting. The arrows indicate the time of appearance of the impulses (8th and 10th) which determine the maximum frequency. After Hartline (15).

energy ($I \cdot t$) necessary to produce a constant amount of excitation (maximum frequency = 56 impulses per sec.) is plotted as a function of duration of flash (t). It is seen that the reciprocity law holds for the single sense cell for all

durations less than, in this case, 0.2 sec. It would appear, then, that the primary photochemical process is relatively simple, or at least that any complicating reactions such as might interfere with the exact application of the reciprocity law are slow, compared with the durations here involved.

In Figure 3 the particular feature of the sensory discharge which is used to test the reciprocity law is the maximum frequency attained. Now it is to be noted, in Figure 1, that with prolonged exposure the frequency in the initial burst of impulses reaches a maximum in a certain length of time, after the onset of illumination. Durations of exposure which exceed this time cannot, of course, affect this feature of the response. Thus the very nature of the response restricts the range of durations which logically may be considered in reference to any such feature as, say, maximum frequency. If, for any reason, this restriction is unrecognized or ignored, the reciprocity law will appear to fail for all times of exposure which exceed a certain critical duration. This, of course, is through no failure of the reciprocity law for the actual photochemical part of the process.

For exposures which exceed the critical duration, only intensity can affect the particular feature of the response which is being observed, and for constant response the condition $I \cdot t = \text{const}$ will be more or less abruptly superseded by the condition $I = \text{const}$. Indeed, in a less direct observation of sense organ activity, such as a reflex response or a subjective report, the only clue to such a logical restriction might be this break in the data. In plotting Figure 3, this logical restriction has been ignored, and it is seen that the data do, in fact, exhibit a critical duration at 0.25 sec. above which the reciprocity law is superseded by the relation $I = \text{const}$ (inclined line with slope of unity). In the experiments of Figure 3, however, the impulses determining the maximum frequency (eighth to tenth) occurred at *ca* 0.5 sec. (indicated on Figure 3 by the arrows). The break in the data thus occurs at an earlier time than the actual appearance of the impulses. It is a plausible step to explain this observation by assuming that the determination of this feature of the response, within the sense cell, precedes the actual appearance of the impulses by an appreciable time. The interval between critical duration and time of appearance of impulses is presumably occupied by processes intervening between the initial photochemical activity and the final nerve response.

Within the range of durations which may be considered in the above experiments, the photo-sensory process exhibits no significant deviation

from the reciprocity law. This, however, cannot be true over a more extended range of exposure times, for Hecht (18) has pointed out that the processes underlying the phenomena of dark and light adaptation necessarily complicate the simple photochemical reaction. The recovery of sensitivity of a photoreceptor during dark adaptation may be ascribed to the reversible, or pseudo-reversible nature of the photolysis of the photosensitive substance. The "back" reaction which replenishes this substance out of the products of photolysis takes place regardless of the state of illumination; it consequently opposes the photochemical breakdown and, for indefinitely long exposures, results in the stationary state, characteristic of the completely light adapted eye. It is obvious that in such a system the reciprocity law can only hold for short durations of exposure.

The back reaction, serving to replenish the supply of photosensitive substance as it is decomposed by light, is a very important feature of the visual photochemical system; its role in the visual process has been emphasized by Hecht (18). The reduction in sensitivity of the photoreceptor, following prolonged exposure to light, and its subsequent recovery during dark adaptation are readily shown in the single sense cell of the *Limulus* eye. A test flash of given energy is presented at intervals following a period of light adaptation, and either the frequency or the total number of impulses in the response is taken as a measure of the sensitivity of the sense cell. In

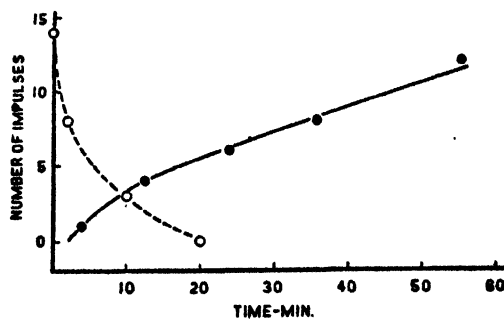


Figure 4. Dark and light adaptation of a single visual sense cell from the eye of *Limulus*. The solid circles give the number of impulses (ordinates) discharged in response to a constant test flash at various times (abscissae) after the eye has been returned to darkness, following 20 minutes of illumination. The open circles give the number of impulses obtainable immediately after periods of illumination of various durations (abscissae). See text.

Figure 4, is given a curve (solid) showing how the total number of impulses in response to a test flash gradually increases as dark adaptation

progresses. This curve was obtained following complete light adaptation (exposure of 20 minutes) at a given intensity. If the light adaptation is incomplete, only an upper portion of the curve is obtained, and by extrapolating back to the instant the adapting light was turned out, a measure of light adaptation is obtained. The course of light adaptation may thus be followed; the "dashed" curve of Figure 4 is an example. In this case the ordinates are numbers of impulses in the response obtainable at the instant the adapting light is turned off, and the abscissae are the durations of exposure of the adapting light. This experiment shows unmistakably the general trend of both dark and light adaptation.

The photochemical part of the mechanism of photoreception is comparatively well understood. Little, however, is known of the processes which follow as a result of the initial photochemical activity, and which eventually end in the production of nerve impulses. The very fact that an interval of time exists between the onset of illumination and the appearance of the response is of significance, and the marked dependence of this latent period upon intensity has led Hecht to postulate, as the second step in the chain of events, a reaction catalysed by the products of photolysis.

Attention has already been called to the interval between the critical duration for a given feature of the response and the final appearance of the impulses, and it has been pointed out that this interval is undoubtedly occupied by processes intervening between the initial photochemical reaction and the final response. A study of various features of the response, with reference to this interval and to the critical duration, and an investigation of the effects upon them of such factors as intensity, dark adaptation, temperature, *et cetera*, will doubtless greatly increase an understanding of these intermediate processes.

In connection with the final step in the process of sense cell activity—the actual initiation of the nerve impulses—it is of interest to consider one of the striking objective phenomena associated with the excitation of photoreceptors. This is the retinal action potential, a large, relatively slow change in potential which can be detected in any of the higher animals, upon illumination of the eye. It has been extensively studied (cf. Kohlrausch (20)), and is certainly intimately connected with the process of photoreception. Of particular significance in the present discussion is the direction of this potential change with respect to the orientation of the sense cells. In general it may be said that, regardless of the structure of the retina in various types of eyes, the major part of the retinal potential is in such

direction that the free, distal ends of the sensory elements become negative with respect to the layer of the retina containing the nerve fibers. The local currents thus set up, surrounding the sense cells and their nerve fibers, are in the same direction as the local currents associated with the nerve impulse which, according to the Bernstein-Lillie theory of nerve activity, are so important in its propagation. The local action currents arising in the visual sense cell are thus in the right direction for initiating impulses in its attached nerve fiber. The hypothesis which this observation suggests then, is that the photochemical action of light ultimately results in a depolarization of the sense cell which is recorded as the retinal action potential, and which gives rise to local currents directly responsible for the production of nerve impulses in a manner entirely in keeping with present day views as to the nature of the nerve impulse. This hypothesis is of general applicability; it was brought forward by Adrian and Buytendijk (4) in their study of the potentials observed in the brain of the gold fish, and has been discussed by Davis, Derbyshire, Lurie and Saul (11) in connection with cochlear and auditory nerve potentials.

Attractive as this interpretation of the role of the retinal potential may be, it cannot escape certain objections. In the first place, there is some question as to whether the retinal potential originates in the sense cells themselves in certain of the eyes which have been studied. Thus the nervous layer in the vertebrate retina, and the large optic ganglion in the insect eye may contribute largely to the observed potentials. These cases would be more analogous to the brain potentials of the gold fish, as has been pointed out by Adrian and Buytendijk. There are, however, at least two cases in which this criticism cannot be brought to bear. In the cephalopod eye the ganglion is a separate structure, and there is little else than sensory cells and nerve fibers which could give rise to action potentials (Beck, (7); Piper, (22); Fröhlich, (12); cf. Kohlrausch, (20)). The eye of *Limulus* is totally lacking in ganglion cells (Grenacher (14)); it is possible, moreover, since the eye is coarsely faceted, to isolate a single one of the ommatidia, containing about 12 to 16 sense cells, and obtain retinal potentials from it (unpublished experiments). The slightest injury to the sense cells, as that caused by inserting the point of a fine needle into the structure, is generally sufficient to obliterate all trace of the retinal potential.

This preparation of a single ommatidium from the eye of *Limulus* is well suited for comparing the retinal potential from a few elements with the discharge of impulses in a nerve fiber from

one of them, for the delicate bundle of nerve fibers, several tenths of a millimeter long, from the sense cells of an isolated ommatidium may be dissected out with the rest of the structure. The retinal potential of the unit is recorded from leads to the cornea and to the back of the ommatidium; by including the nerve bundle in the circuit, nerve impulses, frequently in a single one of the fibers, may be recorded superimposed on the retinal potential.

Such a record is shown in Figure 5. It is seen that as the base-line rises, in the slow retinal



Figure 5. Action potentials from an isolated ommatidium (12 to 16 sense cells) from the eye of *Limulus*. Leads from the attached piece of cornea to the cut end of the bundle of nerve fibers emerging from the back of the ommatidium. The slow rise is the retinal action potential; the spikes are action potentials due to impulses in the nerve bundle, which has been cut down so that only one fiber is active. (The variations in size of the spike potentials are due to failure of the nerve at high frequency and with prolonged activity; cf. Hartline and Graham, (16)). The time marker beats 1/5 seconds, above its record is the signal marking the period of illumination.

potential, the frequency of nerve impulses increases, and has its maximum value approximately at the top of the retinal potential maximum, falling again to a lower value as the retinal potential subsides. In the light of the hypothesis we are considering, this is due to a progressive rise and fall in the depolarization of the sense cell (cf. Adrian and Buytendijk (4)).

While it is true that in general the optic nerve discharge parallels the retinal potential (Kohlrausch (20)) a closer analysis of records such as represented in Figure 4 shows clear-cut discrepancies. For example, a comparison at a given level of potential of the impulse frequencies on the ascending and descending limbs of the retinal potential may show considerable differences, although the amount of depolarization of the sense cell, as indicated by the level of the retinal potential, is the same. Particularly noticeable is the absence, in the retinal potential, of anything corresponding to the marked dip in frequency following the initial burst of impulses (Figure 1), a feature typical of the sensory discharge ("silent period", Hartline and Graham (16)). Moreover, under different conditions of light adaptation the relation between retinal potential and impulse frequency may vary considerably. The difficulties raised by these observations may be by no means insurmountable, but at present it must remain an open question whether the retinal potential is but one of the by-products of photoreceptor activity, or whether it stands in a direct causal relation to the impulses in the sensory nerve fiber.

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DISCUSSION

Dr. Wald: In what way do the responses in single optic nerve fibers from the vertebrate eye differ from those in *Limulus*?

Dr. Hartline: Responses in single optic nerve fibers dissected from the anterior surface of the frog's retina differ from the *Limulus* responses principally in the diversity of their behavior. All

the single fiber preparations from the *Limulus* eye show responses which are essentially the same as those figured here. In the frog eye, however, quite different responses are obtained, even in fibers from closely adjacent regions of the same retina. Certain of the fibers show an impulse discharge closely similar to that found in *Limulus*; in the majority of them, however, it is quite different. Thus the initial burst of impulses may be completely absent, the discharge building up slowly to its steady value. Other fibers show only a short burst of impulses at the onset of illumination and no maintained discharge. Both of these types of fibers may give a burst of impulses when the light goes off. Finally, there is a group of fibers which show no activity whatever during illumination, but which give a vigorous burst of impulses in response to its cessation. This latter type of discharge may subside quite slowly, but is at once suppressed if the light is turned on again, to reappear as soon as the light goes off.

Dr. Wald: Upon what evidence are such differences ascribed to lateral interaction among the receptor elements or their afferent connections in the vertebrate retina?

Dr. Hartline: The very diversity of response among the fibers from the frog retina is indication that we are not dealing with pure sensory activity, but that the nervous elements of the retina have introduced additional complexity. That adjacent pathways can influence each other to a certain extent is indicated by preparations containing several fibers of the last type referred to above. Following prolonged and intense illumination, such fibers may give an off effect which is much prolonged, and which subsides very slowly. It is frequently the case that under such conditions the discharge breaks up into rhythmic bursts of impulses, and when this happens it can be seen quite clearly that the bursts of impulses in the several individual fibers are synchronous. Direct evidence, from experiments on single

fibers, of interaction of the type described by Adrian and Matthews is still lacking. In the *Limulus* eye, which lacks anatomical interconnections, Graham has shown that the discharge of impulses from a given sensory cell is not affected by the activity of adjacent receptor elements. This same experiment should be performed on single fibers of the vertebrate retina, as an extension of Adrian and Matthew's original investigations.

Dr. Hecht: I notice that you have not said anything about the differences in the visibility curves of different sense cells.

Dr. Hartline: The visibility curves of different visual sense cells of the *Limulus* eye agree in their general features, but do show small though probably significant differences. These differences may amount to 0.9 log units at the extremes of the spectrum, even in cells from the same eye. The reproducibility of the determination of visibility is always better than 0.2 log units. The cause of these differences is not known, but their presence results in a differential sensitivity to wavelength among the receptor cells of the *Limulus* eye, which may be considered a peripheral mechanism for color vision.

Dr. Castle: What are the time relations between the beginning of the retinal potential and the appearance of the first nerve impulse?

Dr. Hartline: In those experiments on isolated ommatidia where there is no spontaneous discharge of impulses, the retinal potential always begins before the first impulse appears.

Dr. Castle: Is it possible to obtain retinal potentials at intensities which are below the threshold for impulses?

Dr. Hartline: On the contrary, the retinal potential always disappears, or at least becomes so small that it cannot be recognized above the level of amplifier noise and small spontaneous baseline drift, at intensities which can still produce a fair number of nerve impulses.

THE CHEMISTRY OF THE VISUAL PURPLE SYSTEM

GEORGE WALD

Biological photoprocesses appear to be universally associated with the presence of carotenoids. The significance of this relation has not heretofore been demonstrated in a single instance. The association itself has been revealed repeatedly in the photosynthetic systems of purple bacteria (Schneider, 1930), algae, and higher plants (Palmer, 1922); the eyes of various crustacea (Lwoff, 1927); the all-cone retinas of certain birds and reptiles, and the mixed retinas of frogs (Capranica, 1877; Kühne, 1878).

Recently the retinas and pigmented layers of the eyes of frogs and several mammals have been shown to contain large quantities of the carotenoid, vitamin A (Wald, 1933). This is peculiarly significant in view of the functional relation which exists between vitamin A and vision. Animals deprived of the vitamin—or its carotenoid precursors, the carotenes and kryptoxanthin—become night-blind: when they are transferred from bright to dim light, their retinas fail to increase in sensitivity, or do so abnormally slowly. Figure 1 shows the slow and incomplete

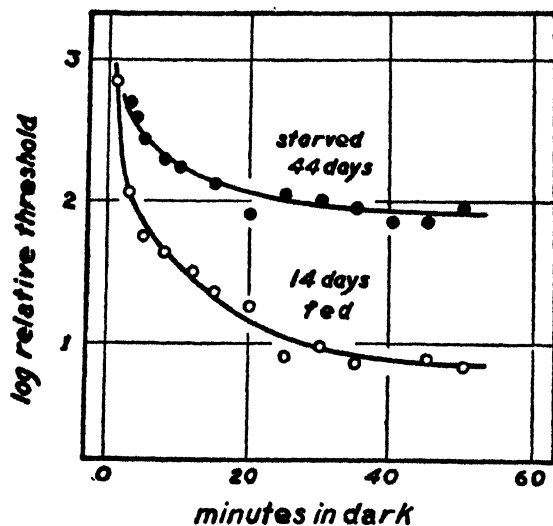


Figure 1. Closed circles: The dark adaptation of a hospital patient after 44 days without food. Optimal color discrimination and visual acuity—cone functions—were normal. Open circles: Dark adaptation of the same person 14 days after feeding had been resumed and weight was again normal. (After Kravkov and Semenovskaja, 1934).

dark adaptation of a starved human subject, compared with the same person's adaptation after he had again attained normal weight (Kravkov and Semenovskaja, 1934). Fridericia and Holm (1925) and Tansley (1931) have demonstrated by the direct analysis of rat retinas that this abnormality is due to failure to synthesize visual

purple. The present paper discusses the mechanism of this relation.

The retinas of dark adapted frogs may be extracted thoroughly in the dark with benzene without injuring their visual purple content. The benzene extracts are colorless, and contain a very small quantity of vitamin A, detected by the absorption band at about 615 $m\mu$ which this substance yields when treated with antimony trichloride. The same retinas may be extracted subsequently with chloroform, a reagent which quickly destroys visual purple. The chloroform extracts are greenish yellow in color, and contain large quantities of the carotenoid, retinene, distinguished in the antimony trichloride reaction by an absorption band at about 660 $m\mu$ (Wald, 1934). The results of a typical experiment are shown in Figure 2.¹

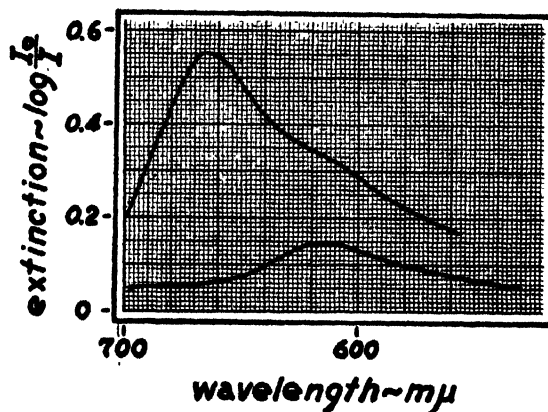


Figure 2. Absorption spectra of the antimony trichloride reaction with a benzene extract of dark adapted bull-frog retinas (lower curve) and with a subsequent chloroform extract of the same retinas (upper curve). The benzene extract contained vitamin A alone, the chloroform extract retinene and an additional trace of the vitamin.

Retinene is readily soluble in benzene after extraction from the retina, yet can not be extracted so long as the visual purple remains intact. Its liberation on destruction of the latter pigment suggests that in the dark adapted retina it is bound within the visual purple complex.

Retinas from frogs which have been exposed to bright daylight for one-half hour or longer are colorless, and yield colorless extracts which contain no retinene. Their vitamin A content, how-

¹The spectra shown in Figures 2 to 4 were measured with a recording photoelectric spectrophotometer developed by Professor A. C. Hardy at the Color Measurements Laboratory of the Massachusetts Institute of Technology. The curves were drawn by the instrument itself, and have merely been mounted and photographed.

ever, has risen from the trace present in the dark adapted retina to about 0.25 γ per eye (*R. pipiens* or *esculenta*). Experiments with isolated retinas reveal the mechanism of these changes.

Isolated dark adapted retinas exposed to bright light turn quickly to a bright orange color (visual yellow). In this condition they yield their full content of both vitamin A and retinene to benzene. The bleaching of visual purple by light therefore liberates retinene, just as does its destruction by chemical means.

Isolated retinas, extracted immediately after bleaching, yield about the same quantities of vitamin A and retinene as do dark adapted retinas. If, however, bleached retinas are left at room temperature, the visual yellow fades and within about an hour has vanished. Extracts of wholly faded retinas are colorless, and contain no retinene. They do contain a large quantity of newly arisen vitamin A (Figure 3). Extracts of partly faded retinas contain intermediate quanti-

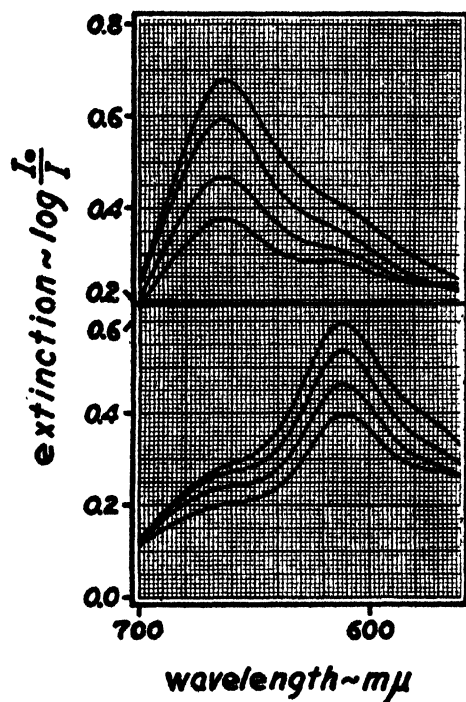


Figure 3. Spectra of the antimony trichloride reaction with chloroform extracts of dark adapted bull-frog retinas (upper series), and of isolated retinas from the same animals which had been bleached and allowed to fade for about an hour in the light at room temperature before extraction (lower series). In each series the spectra were recorded at successive intervals after mixing a sample of extract with antimony trichloride, and follow for about 15 minutes the fading of the blue color produced in this reaction. The dark adapted retinas yield a large quantity of retinene and little vitamin A, the bleached and faded retinas the reverse.

ties of both carotenoids. The fading process converts the retinene liberated by light into the vitamin A found in light adapted retinas.

The fading of bleached retinas is an ordinary thermal reaction. It occurs in darkness with about the same velocity as in the light (Figure 4). It is delayed for hours, even in bright sunlight, by cooling the retinas to 0°C.

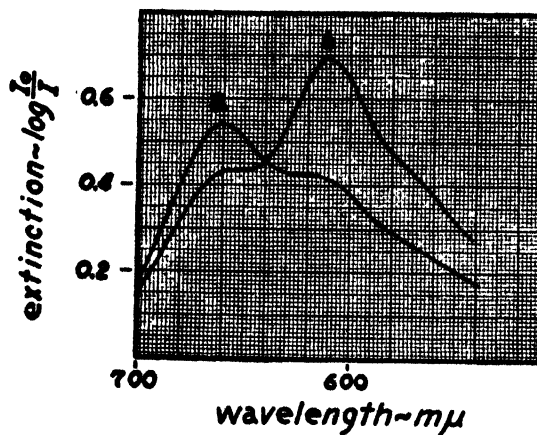


Figure 4. Spectra of the antimony trichloride reaction with extracts of isolated paired bull-frog retinas which had been replaced in the dark immediately after bleaching for 20 seconds in bright daylight. (a) Extracted after 2 minutes in the dark; (b) extracted after 69 minutes in the dark. 20°C. Retinene is converted to vitamin A in the dark about as quickly as in the light.

Isolated bleached retinas, returned to the dark, in addition to fading regenerate some visual purple. The bleaching of visual purple to yellow is therefore a "spontaneously" reversible process.

The colorless products of fading do not regenerate visual purple except in the intact eye. This is the usual dark adaptation process; in it visual purple and bound retinene—which I believe to be identical—are formed as free vitamin A is removed. The latter substance appears therefore to be the precursor of the former. That vitamin A is the visual purple precursor is further demonstrated by (a) the absence of all other carotenoids from the light adapted retina; since higher animals apparently cannot synthesize carotenoids *de novo*, and vitamin A is a product of visual purple decomposition, it must be its own ultimate precursor; and (b) the failure of visual purple synthesis (night-blindness) in animals deprived of the vitamin. The visual processes therefore constitute a cycle.

Except for the synthesis of visual purple from vitamin A, the isolated retina appears to reproduce faithfully the processes which occur in the intact eye. In both cases the initial product of bleaching is visual yellow (Ewald and Kühne,

1878, III, p. 395; Holm, 1923). In continuous illumination both situations end in colorless retinas which contain vitamin A alone. However, isolated retinas which have been bleached and entirely faded contain 2 to 4 times as much vitamin A as those which are light-adapted in the animal. The essential difference between these situations is that in the isolated retina vitamin A is formed irreversibly; while in the intact eye it appears as part of a continuous cycle within which it is formed and re-formed repeatedly during light adaptation. It must be assumed that some vitamin A is lost in this process. This accounts for the dependance of the visual purple system upon a continuous supply of vitamin A in the diet.

The chemical behavior of visual purple (Ewald and Kühne, 1878, IV) is typical of a well-defined group of animal and plant pigments, the carotenoid-proteins. These, like visual purple, are all non-dialysable and may be salted from aqueous solution. They are destroyed by warming, mineral acids and alkalis, acetone and alcohols. Treatment with any of these agents liberates the carotenoid, whose color replaces that of the complex (Verne, 1926, p. 27). This is precisely the relation between visual purple and retinene. Visual purple behaves as a conjugated protein in which retinene is the prosthetic group.

The visual cycle in the frog appears therefore to possess the following components: Visual purple is dissociated by light into retinene plus protein (visual yellow). The retinene is removed by thermal processes in two directions: (a) reversion to visual purple; and (b) decomposition to vitamin A. In the intact eye vitamin A is resynthesized to visual purple, completing the cycle.

I have had little opportunity as yet to test by comparative studies the generality of this solution. The presence of vitamin A in the retinas of pigs, sheep and cattle (Wald, 1933) is significant, since mammalian visual purple is spectroscopically identical with that of frogs (Köttgen and Abelsdorff, 1896). The isolated retinas of cattle, kept dark after slaughtering, have been examined directly and after bleaching and fading—processes which follow the same course as in the frog. Those examined before bleaching contain a small amount of benzine-extractable vitamin A and large quantities of chloroform-extractable retinene. Bleached and faded retinas contain a large amount of vitamin A alone. The visual purple system of cattle is thus chemically indistinguishable from that of frogs.

Köttgen and Abelsdorff (1896) showed the visual purple of fishes to be unique in possessing an absorption maximum at about 540 m μ , while that of mammals, birds and frogs is at about

500 m μ . Nevertheless, I have found the retinas of the common sea robin (*Prionotus carolinus* (Lin.)) to exhibit properties in bleaching and fading similar to those of the frog, and vitamin A and retinene occupying the same positions in the visual system.

The all-cone retina of a turtle, *Clemmys insculpta*, has also been examined. This offers an interesting problem, in that nothing whatever is known at present concerning the chemistry of cone vision. It is significant that in mammalian night blindness due to vitamin A deficiency, the cone function remains relatively unimpaired. The characteristic oil droplets of the turtle retina are colored with large quantities of other carotenoids, but not a trace of vitamin A could be found in this retina. Cone vision is apparently independent of this vitamin. Yet the frequent association of cones with other carotenoids suggests the possibility of some basically similar functional relationship.

It may be concluded that, like other biological photoprocesses, those occurring in the retinal rods are associated with carotenoids—in this case vitamin A and retinene. The vitamin is a simple, though unique, chemical component of the visual cycle.

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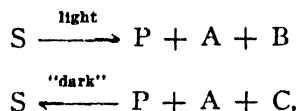
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DISCUSSION

Dr. Mestre: What is the relation between the reactions which you have described and Hecht's photoreceptor equations?

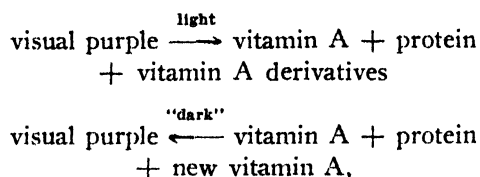
Dr. Wald: Hecht's equations describe the variations in certain visual properties as functions of the concentration of a hypothetical photosensitive substance, S. In the case of the rods, this substance may be identified with visual purple. Hecht has found the bleaching of visual purple to yellow in solution to behave as a simple, first-order photoprocess; and the restitution of the

visual pigment *in vivo*, measured indirectly in dark adaptation experiments, to follow the course of a second-order reaction. Certain general considerations, added to this information, have led him to express the photoreceptor system of the rods in the equations:



in which B is a component removed from the retina very rapidly, and to which the excitation is due; and C is some continuously available addition to the system.

Obviously these equations are simpler than the chemical system which I have described. This follows from their origins, into which reactions in which retinene participated probably did not enter significantly. Nevertheless, one may, without violence to either analysis, substitute for Hecht's equations:



in which the term, "vitamin A derivatives", represents the loss of vitamin A in light adaptation. A comparison of both sets of equations suggests that this term may represent the stimulating material.

Dr. Brackett: Wald's postulation of an immediate dissociation of a carotinoid structure from a protein through the mechanism of light absorption is very interesting in view of certain broad physical considerations. One finds that the shorter the wavelength or the higher the energy of the quantum, the smaller the element of mechanism affected in absorption. Thus in the visible and ultraviolet an electron is generally changed in energy with respect to the general configuration. If this electronic energy is transformed into vibrational energy, a small element, that is a light atom or radical, is most likely to be affected. Consequently the dissociation of two large radicals would seem unlikely unless the immediate electronic mechanism of binding is directly affected in the absorption of energy. Is there any evidence that this is the case?

Dr. Wald: The specific absorption of retinene is in the ultraviolet; its spectrum shows a first small inflection at about 405 mμ. The visual purple maximum is at about 500 mμ. This large change in spectrum must be due to the optical properties of the retinene-protein bond itself. The region of the bond is thus the locus of light absorption. Little or no internal redistribution of

the absorbed energy is therefore required to make it available for dissociation of the molecule at this point. Indeed, the dissociation may occur simultaneously with the act of absorption. The photolysis of visual purple thus appears to be a simple, primary process. This conclusion is consistent with Hecht's analysis of the bleaching of visual purple in solution.

Dr. Davenport: One thing troubled me when you were demonstrating the very close relation between vitamin A and night blindness. Do you think the association is as 100 per cent complete as you suggest, or is the relation more complicated? Despite the fact that night blindness is rather rare, families which have been studied, especially in Europe, show six, eight or ten generations affected with scores of night blinded individuals in the family. It has been believed that a genetic factor is responsible, in part, for the result. May we think that in such families the threshold of the amount of vitamin A that is necessary is very much higher than in the ordinary run of people?

Dr. Wald: Night blindness resulting from vitamin A deficiency is clearly distinguished from the hereditary type. The former is highly variable in the diseased individual, due to its dependence on the diet; it does not appear to be correlated in any way with the genetic constitution; it is never complete except in later stages, when it is accompanied by profound degenerative changes in the eye and other organs; it is only one, perhaps the first, of a large complex of symptoms associated with avitaminosis-A. The hereditary form of night blindness, however, consists in the total suppression of rod function in an otherwise normal individual. It is stationary throughout life, and responds to no known treatment. Its mechanism is unknown. There is no reason to believe it correlated in any way with the vitamin A metabolism.

Dr. Moyer: Might not one immunize some animal to visual purple and so possibly produce antibodies to vitamin A and retinene when these are injected alone (so-called hapten reactions)? This should demonstrate conclusively the linkage of these carotenoids to protein in the visual pigment.

Dr. Wald: This experiment has been urged on me repeatedly by Dr. Gregory Pincus, but we have not yet tried it.

Dr. Chase: Have you worked with visual purple solutions, to determine whether retinene and vitamin A can be obtained from them?

Dr. Wald: I have prepared digitonin extracts of dark adapted frog retinas. These yield at most a faint trace of vitamin A to benzene. After bleaching, they yield a large quantity of retinene to any of the common organic solvents. I have not observed any appreciable conversion of retinene to vitamin A in such solutions.

AN ANALYSIS OF THE VISUAL CAPACITY OF THE BEE'S EYE

ERNST WOLF

I

For the experimental analysis of the functions of the compound eye of Arthropods a particular method has been used which so far has given us information about visual acuity, intensity discrimination, critical flicker frequency and dark adaptation in different Arthropods. This method of investigation is based upon a forced reaction of these animals to moving stripe systems. Hecht and Wolf (1928-29) used it for the first time in a study of the visual acuity of the honey bee. If a bee is placed on an inclined surface which is illuminated from below it tends to creep straight upward. If now a system of black stripes with equally wide translucent spaces in between, is moved sidewise underneath the bee's creeping surface, the bee is by the slightest motion of the stripe system thrown off its regular course and creeps *against* the motion of the striped pattern. This reaction occurs with great precision every time when the stripes are shifted and thus can be used as a reliable indicator of the bee's visual capacity.

The size of the stripes can be varied and the threshold intensity of light determined at which the bee will give the first noticeable response. We therefore are able to relate, in the usual terms, visual acuity to the intensity of illumination, and give by means of a curve a picture of the resolving power of the bee's eye (Hecht and Wolf 1928-29). If we intended to plot a visual acuity curve for the human eye and for the bee's eye on the same scale, we would hardly succeed, since the bee's visual acuity is about 100 times worse than the human acuity. The same method of test has been used later on by Hecht and Wald (1933-34) for a study on the visual acuity of *Drosophila*, and by Clark (1932) for the fiddler crab.

If we use instead of a pattern made up of black and translucent stripes a system of stripes different in brightness, i.e. one set of stripes having a brightness I and the other a brightness of $I + \Delta I$ we can by adjusting I determine the necessary increase ΔI to produce a threshold reaction in an insect. For the bee and for *Drosophila* intensity discrimination has thus been determined (Wolf, 1932-33 a, b; Hecht and Wald, 1933-34). The results show that the bee's intensity discrimination is $1/25$, and that of *Drosophila* about $1/150$, of the human.

When studying critical flicker frequencies in relation to illumination it is necessary to move a striped pattern underneath the bee's creeping plane at a certain constant speed so that by the number of stripes passing by per second a certain flicker frequency is obtained. At low frequencies

the light intensity for threshold reaction is low; as the flicker frequency is increased the illumination must be increased. The maximum flicker frequency which can be perceived by the bee's eye is about 55 per second (Wolf, 1933-34). Sälzle (1932) found by a slightly different method for the larvae of Dragon flies (*Aeschna*) a maximum value of about 60 flickers per second. The values for the bee and the Dragon fly compare very favorably with the human eye, for which the maximum flicker frequency varies between 45 and 53 flickers per second (Hecht, Schlaer and Verrijs, 1933-34). It certainly is noteworthy that in regard to visual acuity and intensity discrimination the eyes of the arthropods investigated are far behind the human. The number of single impressions, however, which can be perceived per second is just as great as for man.

For a study of dark adaptation of an insect's eye the method mentioned did not seem very successful. During dark adaptation it is essential that determinations of threshold intensities for response are made rather rapidly. The time required for a bee to creep up an inclined surface and the time necessary for the observer to move the stripe system back and forth, and to determine the threshold amount of light for reaction, unbalances the state of adaptation so much that no accurate readings can be obtained. The method of test has therefore to be altered slightly. A bee is tied into a glass tube with its head sticking through a thin rubber membrane. If a stripe system is now moved in front of it a reaction of the antennae can be noticed. The antennae might be quiet or moving; as soon as the stripes are shifted to the right the antennae at once take a definite almost rigid position. The left antenna points at an angle of 90° to the axis of the bee's body while the right antenna is extended straight forward. The two thus include an angle of 90° . As soon as the motion of the pattern is reversed, the antennae take the reverse position. The reaction occurs with certainty, and each time with the reversal of motion of the pattern. By determining threshold light intensities for this reaction at different intervals in darkness a curve describing the course of adaptation can be obtained for the bee's eye (Wolf, 1935-36).

From all our experimental results one fact is evident, namely that the single receptor element, or groups of receptors, in the bee's eye must undergo a sudden differential change of excitation to obtain a response from an animal. By the shift of the pattern in front of the bee's eye a certain number of elements will experience a transition from light to dark and from dark to light. We

may assume that the thresholds for excitation of the ommatidia of the eye are distributed at random over the whole surface. At low intensities the elements which will be stimulated lie far apart from each other. The higher the intensities the smaller the distance between functioning elements. Keeping this in mind we have no difficulties in understanding the relationships between light intensity and the different visual functions.

II

Without doubt the visual capacity of the bee's eye seems very low as compared with the human eye. Only in regard to flicker is its sensitivity as high as in man. We therefore are justified in assuming that flicker must play a very important role in the bee's vision. This is most apparent in some experiments based upon the bee's positive phototropic response which were carried on in a dark room.

Two holes of equal size are cut into a vertical wall. Into them opal glass plates are fitted which are illuminated from behind. One field is illuminated constantly, whereas the other is flickering at low frequency. Bees which are set free on a horizontal surface some distance away from the two fields will with great certainty migrate to the flickering field, paying almost no attention to the steady field, even if its brightness is increased considerably. The flickering field has a greater stimulating effect than the steady field.

For quantitative studies we arranged a series of five flickering fields of the same size and brightness in one row, to which the bees were allowed to migrate from a starting point 170 cm. from the fields. In front of the light sources illuminating each field there are sector discs which give a ratio of flicker frequencies of 1:2:3:4:5. These are driven by a chain and gear system to keep the flicker ratios the same. If we allow a great many bees to migrate to these fields we find that the numbers of bees "seeking" the different fields are proportional to the flicker frequencies.

When using the same set up mounted in a box outside, and using the side which bears the five openings as top, we can cover the surface with a glass plate and condition the bees to collect a sugar solution which is evenly spread over the glass on top of this "table". While the bees are fed the 5 fields are covered to make the surface look uniform. Bees will willingly return to the experimental arrangement even when they are being brushed off the glass and when the food is being removed. If now the 5 flickering fields are shown, the bees *spontaneously* settle down above the flickering fields. Repeating this test many times one can plot the number of choices among the five fields against their flicker frequencies. Exactly as in the dark room experiment, the

number of choices is directly proportional to the flicker frequencies (Wolf, 1933).

Since the bees' reaction to flickering fields of the *same* area depends upon flicker frequency, it is of interest to see how bees react to steady and flickering fields of *different* areas.

Into a vertical wooden plate, which serves as one wall of a triangular cage, two openings 37 x 37 cm. are cut. Into each opening opal glass plates are fitted which are illuminated from behind by two 500 watt bulbs. The intensities of light falling onto the opal plates can be controlled by diaphragms which are placed in front of the two sources. From the apex of the triangular cage bees are allowed to migrate toward the two illuminated squares. If the intensities of both fields are equal, in a great number of trials equal numbers of bees will go to either field. Each bee is set free singly and takes a course which is the bisecting line between the two fields. As soon as the animal has almost reached the front wall, random motions occur and the chances are equal that the animal turns to the right or to the left. If we now reduce the area of one of the fields to $1/2$, $1/4$, $1/8$, or $1/16$, more and more bees migrate to the larger field. Since the brightnesses of both fields are equal we may assume that the response to the larger field is due to the greater number of elements stimulated, and hence the greater total frequency of retinal responses. However, Hartline and Graham (1932) have shown that the total frequency of optic nerve discharges in *Limulus* also increases with intensity. It therefore seemed possible to equalize the stimulating effect of the two different areas by decreasing the intensity of the larger area, until the total frequency of responses from both areas is equal. In order to test this point we decreased the brightness of the larger field step by step until we obtained, in a considerable number of tests, equal numbers of bees going to both fields. When this is the case the product of area times intensity is identical for both fields (Table I).

Since the larger area stimulates a greater number of receptor elements weakly, while the smaller area stimulates a smaller number more strongly, due to the respective brightnesses, we must assume that it is irrelevant for the processes which control the coordination of motion whether weak impulses travel from a greater number of receptor elements over a greater number of nerve fibers, or whether a smaller number of stronger impulses pass over fewer pathways.

If we present to bees, instead of stationary fields, two flickering fields of which one is flickering at a constant frequency and the other at an integral multiple of this frequency, can these two fields be balanced in their stimulating effect by adjusting their areas?

TABLE I

Values for areas and intensities of illuminated fields which have the same stimulating effect upon the bee's eye. The products of area times intensity are approximately equal for two fields presented simultaneously.

Area cm.	Intensity Millilamberts	Area x Intensity	Area cm.	Intensity Millilamberts	Area x Intensity
1354.2	6.42	8696.9	1354.2	6.42	8696.9
681.2		4374.5		3.26	4304.8
338.6		2174.2		1.70	2302.2
169.0		1085.3		0.75	1019.7
86.5		555.4		0.54	578.9

At first we make both fields equal in brightness and size, the flicker frequency of one being twice that of the other. The bees now migrate to the faster flickering field. We then decrease the size of the faster flickering field until equal numbers of bees travel to both fields. This point is arrived at as soon as its size is reduced to one half of the original. If the ratio of flicker is 1:4 the respective ratio of areas must be 4:1. In all our tests it can be shown that, for equal photic stimulation by flickering fields, the field areas must be inversely proportional to the flicker frequencies. For the phototropic orientation of bees we can therefore assume that it is irrelevant whether a great number of ommatidia is stimulated at a low frequency or a smaller number more often per unit time.

III

The effect of intermittent stimulation, which can nicely be demonstrated in laboratory experiments, can also be found in nature. First we shall prove this in the case of some reactions of bees to patterns of different design. Just as bees will settle down above flickering fields *spontaneously*, they will, after being conditioned to take food from a glass topped table, settle above patterns which are placed underneath the glass top and to which they were not conditioned previously (Hertz, 1930). By selecting a series of patterns of different design and presenting them repeatedly to bees for choice one can obtain information about the distribution of the bees over the patterns. Hertz (1930, 1931, 1933) pointed out that there is a connection between the bee's choices and the "richness of contours" of the patterns. By selecting 9 patterns of different design but the *same* area Zerrahn (1933) found that the number of choices of each pattern is proportional to the length of its contours (length of contours = length of edges black against white within

each pattern). The contours, however, are a measure of flicker produced when a bee flies across a pattern. We therefore can relate these findings with those obtained during our phototropic tests. Let us, for example, take in the extreme case two patterns which are quite different regarding their structure, namely, a black disc and a checkerboard pattern of the same area. It becomes at once apparent that the number of elements which during flight will undergo changes in their state of excitation are quite different. For the black disc only the marginal elements experience a change, while those looking at the center portion will remain under the same condition of stimulation over a longer period. For the checkerboard pattern a great many elements which lie close together undergo changes, and consequently the number of alternating stimuli produced by this pattern is considerably greater than the number produced by the disc. To explain the bees' choices it only remains necessary to assume, in analogy to the dark room experiments, that by the transitions of the retinal elements a forced reaction is produced, and the higher the frequency of flicker the stronger becomes the reaction of the bees.

By presenting to bees two sector-wheel patterns of the same dimensions we obtain equal numbers of choices. By rotating one of the two we are able to increase the number of transitory stimuli. We then find that the choices of the bees turn almost entirely to the rotating pattern (Wolf, 1933).

So far we have been dealing with patterns which were always of the same area. If the bee's reaction depends only upon the number of retinal elements undergoing changes, we ought to be able to balance the effect of two patterns, different in area, by adjusting their "grain" so that both produce the same number of alternating stimuli.

For such tests checker boards and striped patterns were used. First a checker board (10 x 10 cm.) with checkers 1 cm. square is presented, together with other patterns of a coarser grain (1.5 cm. squares, 2 cm. squares, and 4 cm. squares). To obtain equal numbers of choices the 1.5 cm. pattern must have an area of 12 x 12 cm., the 2 cm. pattern must have an area of 14 x 14 cm., and the 4 cm. pattern an area of 20 x 20 cm. The increase in area of the coarser pattern is consequently proportional to the increase in length of the sides of each single square. Since we know that the stimulating value of a pattern is characterized by its length of contours we understand at once the increase in area which is necessary for equal stimulating effects. By the increase in area we just balance the loss on contours which was produced by the coarser design. For all the patterns which have the same stimulating value we

find in fact that the length of contours is approximately 200 cm. Equal numbers of elements could therefore be stimulated alternately during the bees' flight over the patterns.

If we use striped patterns instead of checkerboards the experimental conditions are not quite as favorable, since their stimulating value depends largely upon the direction in which they are crossed by the bees. With a sufficiently great number of trials, however, results can be obtained which are comparable to those secured with the checker boards. For comparison all the data are summarized in Table II.

now the pattern is moved over a unit length of distance in any direction, the number of elements undergoing transitions during the shift may be counted. For any coarser pattern which does not provide as many transitions as a fine one of the same area when moved over the same distance, the increase in size which would give it a stimulating value corresponding to that of the fine pattern can be estimated. We started our investigation, in fact, by studying the effect of moving patterns upon the elements of this model of the bee's eye, and the calculations made were justified by the results of our experimental tests.

TABLE II

Summary of the results of the bees' choices among patterns different in area and coarseness of design.

Exp. No.	No. of Tests	Pattern	Area cm	Length of Contours cm	No. of Choices	Pattern	Area cm	Length of Contours cm	No. of Choices	Choices of both Patterns	Ratio fine:coarse
Checker-board						Checker-board					
I	51	1 cm ² (A)	10 x 10	200	31	2 cm ² (B)	10 x 10	104	11	9	2.8:1
II	54	" "	" "	" "	21	2 cm ² (C)	14 x 14	200	21	12	1:1
III	53	" "	" "	" "	14	2 cm ² (D)	20 x 20	400	30	9	1:2.1
IV	54	" "	" "	" "	29	1.5 cm ² (E)	10 x 10	142	18	7	1.6:1
V	53	" "	" "	" "	26	1.5 cm ² (F)	12 x 12	192	26	1	1:1
VI	75	" "	" "	" "	53	4 cm ² (G)	10 x 10	64	16	6	3.3:1
VII	60	" "	" "	" "	27	4 cm ² (H)	20 x 20	208	27	6	1:1
Stripes						Stripes					
VIII	53	1 cm (I)	10 x 10	110	31	2 cm (K)	10 x 10	72	17	5	1.8:1
IX	50	" "	" "	" "	23	2 cm (L)	12.5 x 14	116	23	4	1:1
X	52	" "	" "	" "	15	2 cm (M)	20 x 20	220	32	5	1:2.1

A picture of the effect of the alternate stimulation upon the bee's eye during motion, when looking at a pattern, can be provided by mapping out on a sheet of translucent coordinate paper the points of intersection of the axes of the ommatidia with a plane whose distance from the eye corresponds to the distance of the pattern at the moment of choice. This distance is in our case approximately 15 cm. Such a system of coordinates can be obtained from a figure given by Baumgärtner (1928) which represents a schematic picture of the resolving power of the bee's eye. His coordinates only need to be replotted for our distance. By placing our patterns underneath the map of the bee's eye, one can count the number of elements which are covered by the white and by the black parts of the pattern. If

IV

When looking at insects in nature during their flights, which are devoted mainly to the search of food, we can also explain their visual reactions to flowers as forced reactions to flicker. Most flowers which are visited by bees do not stand singly, but in groups. One only needs to remember blooming fruit trees, shrubs, umbelliferous plants, or single blossoms, like clover, which are cultivated in fields. If a bee during its flight crosses such a flowering surface the elements of the eye experience a great many changes in their state of excitation which may cause a forced reaction resulting in a settling down of the bee. Such a reaction could facilitate the finding of small flowers closely spaced, even when the par-

ticalars of their flower formation are below threshold visibility.

We were anxious to find some confirmation of this theory and have found already, by observing bees visiting flower beds on quiet and windy days, that the number of bees settling down on the flowers is greater when the blossoms are moved slightly by the air current.

Lately we have tried to obtain further proof for the bee's reaction to flicker while collecting on flowers. We used arrangements of natural and artificial flowers to which the bees were conditioned. Sections of our flower beds can be set into motion to produce flicker. We know so far that the moving objects attract more bees than the quiet ones. A clear cut quantitative proof, however, still remains to be given.

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DISCUSSION

Dr. Harris: There must be a limit to the contour "law". Is that limit dependent wholly upon coarseness of the pattern or its total size?

Dr. Wolf: There is a limit to the size of a pattern which can be seen by the bee at a given distance. If the pattern is so big that it covers an area bigger than the area which can possibly be taken in by the total number of ommatidia of the bee's eye, the marginal portions of the pattern certainly cannot have any effect upon the bee's reaction. One must however keep in mind that the bee is moving above the pattern at the moment of choice and therefore the extent of the pattern can produce a greater number of transitions of retinal elements from one state of excitation into another and in that respect a bigger area can have a greater stimulating effect. Probably a relatively small pattern, however, can cause already a sufficient amount of alternating stimulation so that the

bee is forced to settle down. If the bee actually sits on the pattern it will be so close to the pattern that it can see only a very small portion of it.

Dr. Harris: How do bees find a sugar solution in the first place when it is put on top of a table?

Dr. Wolf: To condition the bees to come to a feeding place is a complicated technical affair. I found it easiest to go to the hive and put strips of paper outside, on which is put sugar or honey. The bees will take food from here and, while they are collecting, one picks up the paper with the bees, puts it into a box, closes the box and takes the bees to the experimental arrangement. The bees are left in the box for a while. When they are set free they have to start from that place for their home. According to experiments by Opfinger, orientation to a feeding place occurs after the bees have taken food and started for the hive. Consequently the transportation from the hive to the feeding place does not mean anything for the bees' orientation. By associating the new locality with "food" the bees will afterwards return regularly to our experimental arrangement. One does not always succeed when taking bees from the hive for the first time, but after having taken bees to the place desired several times, very soon a regular stream of visitors is secured.

Dr. Ponder: Are they able to see where they are going when they are being transported?

Dr. Wolf: No, they can be transported in a dark box. It is important, however, that the place to which the bees are taken lies within the hunting ground of the bees of the hive used. The bees must know the surroundings to enable them to return home.

Dr. Ponder: Do you mean that they orient near the feeding place visually?

Dr. Wolf: Yes. If you took the bees in the dark box from the hive to a place where they have never been before and open it there, the bees would go up into the air, describe circles trying to get home, but since they have no known marks for their orientation they return to the box, whereas within a field with which they are acquainted they would just go off.

Dr. Giese: How large is that hunting area?

Dr. Wolf: A circle of about one mile and a half in radius.

Dr. Ponder: Do bees have a sense corresponding to that of smell by means of which they might orient themselves?

Dr. Wolf: There has been done very extensive work on the sense of smell in bees by von Frisch. He has investigated the bees' olfactory discrimination and the distances over which bees are able to perceive specific odors of flowers, and we know that odors play an important role in orientation. To give one example; in the Botanical Garden at the University of Munich hundreds

of different kinds of flowers are planted systematically. Von Frisch conditioned certain bees somewhere else to one specific odor of one species which was in blossom. The bees were marked during conditioning. It was found that these conditioned bees were able to find that specific flower later, when they were placed in the garden. For comparison with our olfactory capacity one might say that the bee's power of olfactory distinction is not greater than ours. For instance, by presenting to them the etheric oils of different species of oranges they were not more capable of distinguishing them than we are.

Dr. Hartline: The reciprocal relation between area and intensity demands explanation, not only in these experiments, but in those on other types of eyes as well. Thus doubling the area illuminated brings twice the number of receptors into play and hence doubles the number of nerve impulses reaching the centers in unit time. Halving the intensity on any given sense cell, however, does not as a rule halve the frequency of its impulse discharge, nor does it even approximately do so, except possibly over a very narrow range of a particular portion of its intensity-response curve. With decreased intensity, of course, receptors with higher thresholds will drop out completely; moreover with increased area the effects of the additional elements brought into play may not be completely summated in the centers. The relation at best is an approximation and holds only over a narrow range.

Dr. Wolf: The range of intensities and of areas both cover only about one logarithmic unit in our experiments. Under these conditions our results can be understood. It is very probable that our area and intensity relation would not hold if we covered a greater range. On account of the structure of the bee's eye this can hardly be done. With very small areas we finally would have only one element stimulated. By increasing the areas tremendously we would reach another extreme, namely we would soon reach a state in which all elements are concerned, and by making our illuminated fields bigger the number of elements stimulated could not be increased further.

Dr. Mestre: I would like to ask two questions; first, whether the bees are oriented with respect to the light sources before being released and, second, how much random movement is apt to be made before the final choice of path becomes evident.

Dr. Wolf: What we actually did during experimentation was to put the bee underneath a beaker at the end of the cage and to leave it there exposed to the light. Then we let it run by removing the beaker. It is striking how precisely the bees take their course in respect to the two areas and their intensities. Random motions oc-

cur, however, in some cases which are of unknown origin.

Dr. Mestre: I should think that if the bees could be uniformly oriented with respect to the light sources before release, more uniform behavior might be obtained. In this way it might be possible to determine with accuracy the choice of the bee when still so far distant from the light sources that no important increase in the number of ommatidia had yet occurred. Wald and I were talking about a similar problem in connection with Blum's paper and he made the suggestion that this pre-orientation could be readily achieved by releasing the animals through a tube.

Dr. Wolf: I think the angle of orientation to two illuminated areas might well be taken as a measure, if the releasing of the bees is done properly. If the intensities of two fields are not the same the course of the animal is taken right away toward the brighter field. Only in case the stimulating effect of both fields is equal, is the course of the bee the bisecting line between two fields. Naturally under such conditions random motions must occur at the end of the course until the animal has arrived at one of the fields.

Dr. Wald: So far as I know, there is no evidence that variation in the distance between a compound eye and a diffuse source produces changes in brightness. Hecht and Wald (1933) found no change in the threshold to stripes resulting from changes in distance of 300 per cent. This leaves area of the field as the only significant variable as an arthropod approaches, or recedes from, the source of stimulation.

Dr. Blum: That, of course, makes my problem much simpler since we may assume that we are dealing with the geometry of the light field, and disregard differences in intensity at different parts of the field.

Dr. Cole: The visual acuity and intensity discrimination data for the bee were determined experimentally by flicker. Are there data on the bee to show how a threshold for steady light compares with a flicker threshold? Were the visual acuity and intensity discrimination data of the bee's eye compared with the steady light or the flicker data for the human eye?

Dr. Wolf: The comparison of our visual acuity and intensity discrimination data for the bee's eye is made with those for the human eye under steady light conditions. The bee's reaction can only be obtained by the motion of a stripe system, thus we have no means of making a comparison on a different basis. The threshold for excitation by light as such is certainly lower than the threshold intensity of illumination during visual acuity, intensity discrimination and flicker tests, during which the stripes as such must be seen to cause the animal's response.

THE MECHANISM AND KINETICS OF BIOLUMINESCENT REACTIONS

E. NEWTON HARVEY

This discussion of bioluminescence¹ will be restricted to the more important facts concerning mechanism and kinetics of luminescence, which have been ascertained from a study of *Cypridina hilgendorffii*. This animal is a marine ostracod crustacean, which extrudes two granular secretions, produced in separate gland cells, into sea water where the granules dissolve. The mixing of water solutions of the substances in these secretions, luciferin and luciferase, in presence of dissolved oxygen, results in luminescence; salt is not necessary for light production. The luciferin oxidizes to oxyluciferin while the luciferase, an enzyme, remains unchanged. By drying the *Cypridinae* quickly, the photogenic substances can be preserved indefinitely. They have quite different chemical properties and can be extracted from the dried material and separated by appropriate treatment. Luciferin is thermostable but easily oxidized, while luciferase is not oxidized but is destroyed on heating, a difference which has made separation of the substances relatively simple.

MECHANISM

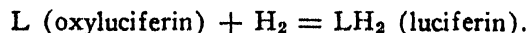
Only if luciferase is present together with luciferin will there be luminescence. On the other hand luciferin in oxygen oxidizes without luciferase, but no light appears. Luciferin is the substrate upon which luciferase acts, but the production is connected with the luciferase rather than the luciferin. This is indicated not only by the fact mentioned above that luciferin when oxidized alone never gives light, but also by experiments on the color of luminescence, by which it can be shown that when luciferase and luciferin from two different forms having different colored luminescences are mixed, the animal supplying the luciferase determines the color of the resulting luminescence.²

If luciferin is oxidized by such agents as $K_3Fe(CN)_6$ in absence of oxygen but in presence of luciferase, there is also no luminescence. Therefore luciferin, luciferase and free oxygen constitute the bioluminescent system and luciferase belongs in the group of oxidizing enzymes. However, no other oxidase can take the place of luciferase and the luciferases of different animals are highly specific, only closely related forms giving luminescence when their photogens are intermixed.

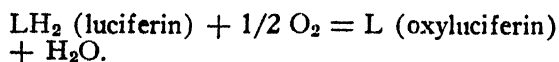
The evidence that luciferase is of enzyme nature is as follows:— first, it exists in water in colloidal solution with the general properties of enzymes; second, it does actually accelerate the velocity of oxidation of luciferin and may be used many times, remaining practically unchanged at the end of the reaction. At the same time it must again be emphasized that luminescence is not merely the consequence of the high velocity of oxidation of luciferin in presence of luciferase. Luciferin without luciferase can oxidize spontaneously at high temperature much more rapidly than with luciferase at low temperatures, but only in presence of luciferase is light produced. It is the presence of luciferase rather than the velocity of oxidation that produces light. However, if luciferase and oxygen are also present, the greater the velocity of oxidation of luciferin, the brighter will be the luminescence. Luciferase plays two rôles, first that of an oxidizing enzyme, accelerating oxidation of luciferin, and second that of a substance capable of excitation to luminesce in a manner to be considered presently.

When luciferin is oxidized, oxyluciferin is formed. This product seems to represent a very slight change in the molecule since it can be partially reduced to luciferin again³. It is not necessary that luciferase be present for reduction to occur, but oxygen must be absent. No luminescence accompanies the reduction.

Since nascent hydrogen is a good reducer, and other methods of reduction involve the taking up of hydrogen, we may suppose some such change as the following to occur:



Oxidation might then be represented as follows:



Among the reducing agents that will reduce oxyluciferin are hydrogen and Pt or Pd; sul-

¹ The following are general articles on light production by organisms. Dubois, *La Vie et La Lumière*, Paris 1914; Molish, *Leuchtende Pflanzen*, Jena 1904 and 1912; Mangold, *Handbuch der vergleichenden Physiologie*, 8, 225, 1910; *Handb. der Normalen u. Pathologischen Physiologie*, 8, (2nd half), 1072, 1928; Dahlgren, *J. Franklin Inst.* 180, 515, 711, 1915; 181, 109, 243, 377, 525, 659, 805, 1916; Harvey, *The Nature of Animal Light*, Lippincott, Phila., 1920; *Physiol. Rev.* 4, 639, 1924; *Bull. Nat. Res. Council* No. 59, 50, 1927; Pratje, A., *Ergebn. Physiol.* 21, 166, 1923; Klein, G., *Handb. der Normalen u. Pathologischen Physiologie*, 8, (2nd half), 1058, 1928.

² Harvey, E. N., *Science* 44, 241, 1917; *Am. J. Physiol.* 70, 619, 1924.

³ Harvey, E. N., *J. Gen. Physiol.* 1, 133-145, 1918; 5, 275-284, 1923.

phides; hydrosulphites as $\text{Na}_2\text{S}_2\text{O}_4$; CrCl_2 ; TiCl_3 ; anthraquinone-2-6-di-Na-sulphonate; anthraquinone-Na-sulphonate; yeast cells and bacteria. Atomic hydrogen will reduce oxyluciferin in the dry state.⁴

The system luciferin-oxyluciferin behaves somewhat as the system leuco-methylene blue—methylene blue and other reversibly oxidizable dyes. Its oxidation-reduction potential has been investigated⁵ by determining which substances having definite redox potentials will oxidize luciferin and which will reduce oxyluciferin in absence of oxygen.

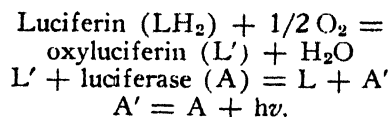
The results indicate that strong oxidizing agents like $\text{K}_3\text{Fe}(\text{CN})_6$ or quinone will oxidize luciferin rapidly, and strong reducing agents like the reduced anthraquinone sulphonates and $\text{Na}_2\text{S}_2\text{O}_4$ will partially reduce newly formed oxyluciferin, but the indophenols will not oxidize luciferin nor will the reduced indigo sulphonates reduce oxyluciferin, which has stood for several hours. Under these conditions the oxyluciferin-luciferin system appears to behave as an irreversible system between an E_0 value of +0.24 to -0.22 volts at pH = 7.7, and we can speak only of an "apparent oxidation potential" of +0.24 volts and an "apparent reduction potential" of -0.22 volts. After standing for some days the oxyluciferin cannot be reduced and secondary changes must occur. No definite redox potential can be assigned to the system.

The light production is undoubtedly a chemiluminescence, light resulting from a chemical reaction, the opposite of a photochemical change. Chemiluminescences may be of two kinds, corresponding to the two varieties of photochemical reactions, (1) the direct photochemical processes in which the molecules absorbing radiation (excited molecules) undergo photochemical change, and (2) the sensitized photochemical reactions in which the photosensitizer absorbs the energy of radiation, later transferring it by collision to other molecules, which undergo photochemical change. Correspondingly, in one type of chemiluminescence, the energy of reaction remains with the reaction product or products (excited molecules), and may be emitted as light; in the second type the excited molecules, resulting from the reaction, transfer by collision their excess energy to other molecules, exciting them to luminescence. The luciferin—oxyluciferin—luciferase system appears to be of the latter type in view of the facts already mentioned, which indicate that luciferase is the source of the luminescence, and that the

color of the light depends on the kind of luciferase present.

We may then suppose that the energy of oxidation of luciferin to oxyluciferin excites luciferase molecules, which luminesce on return to the normal state. Luciferase supplies the molecules capable of excitation, and is in addition a catalyst accelerating the oxidation of luciferin. Luciferase molecules can only be excited by the oxidation of luciferin (and only a particular kind of luciferin) and by no other substances oxidizing in presence of luciferase, although a large number of autooxidative, and other, reactions have been tried. Even recombination of hydrogen atoms, liberating large amounts of energy, gave no certain luminescence when tested in presence of dry luciferase.⁴

A scheme representing the light production of the luciferin—oxyluciferin—luciferase system would be as follows:—



The prime (') indicates an excited molecule possessing excess energy, which is in this case transmitted to luciferase and emitted as a quantum of light. *Cypridina* luciferase emits in a region whose maximum is at $\lambda = 0.8\mu$.

The spectra of luminous animals may then be assumed to represent the emission from the complex luciferase molecule, and it is not surprising to find that these spectra are very broad bands as modern theory requires.⁶

Studies on the quanta of light of $\lambda = 0.48\mu$ produced per molecule of oxygen used in oxidizing *Cypridina* luciferin, show that roughly (an order of magnitude only) 100 molecules of oxygen must react before 1 quantum appears. This means that only 1 in 100 collisions of excited molecules with luciferase are fruitful so far as luminescence is concerned.⁷

Many chemiluminescent substances can be excited to luminesce by exposure to radiation⁸ (fluorescence). Although it has been impossible to excite luminescence of *Cypridina* luciferase (or luciferin) by exposure to ultraviolet light, cathode rays or X-rays, photogenic organs of a number of luminous animals, notably the fire-fly, fluoresce

⁴ Harvey, E. N., and Lavin, G. I., *Science* **74**, 150, 1931.

⁵ Harvey, E. N., *J. Gen. Physiol.* **10**, 385, 1927.

⁶ Coblenz, W. W., *Sci. Pap. Bur. Stand.* **21**, 521, 1926.

⁷ Harvey, E. N., *J. Gen. Physiol.* **10**, 875, 1927.

⁸ Kautsky and Zocher, *Z. Phys.* **9**, 267, 1922; *Electrochem. Z.* **29**, 308, 1923; *Naturwissensch.* **11**, 194, 1923; Kautsky and collaborators, *Z. Elektrochem.* **32**, 349, 1926; *Z. Phys.* **31**, 60, 1925; *Z. anorg. allg. Chem.* **144**, 197, 1925; **147**, 81, 1925.

brightly in near ultraviolet light with a color similar to, but not always the same as, that of the chemiluminescence of the animal. The fluorescence can be observed in the dried animal or after the organ has been boiled so that the possibility of chemiluminescence is definitely prevented.⁹

Luciferin may also undergo photochemical change. The photochemical effect is not the reverse of the photogenic, however. Light hastens the oxidation of luciferin, not the reduction of oxyluciferin. Thus, a luminescent solution of luciferin and luciferase partly exposed to the condensed beam of an arc light will have its luminescence suppressed in the lighted region in a few seconds. It can be shown that this is not due to heat, but is a light effect solely on the luciferin, not on the luciferase. It takes place only in presence of oxygen. The luminescence is suppressed because the luciferin is rapidly oxidized in the light as compared with its rate in the dark. It can also be proved, by the use of photosensitive dyes and proper color screens, that this rapid oxidation of luciferin in light, even though it takes place in presence of luciferase and oxygen, does not result in luminescence. The meaning of this fact is not clear and deserves further investigation, for other experiments all indicate that, in presence of luciferase and oxygen, more rapid oxidation of luciferin means greater luminescence. The photochemical oxidation of luciferin is due to wavelengths in the region $\lambda = 0.45\mu$ to $\lambda = 0.38\mu$. Some fluorescent dyes photosensitize for longer wavelengths. It does not seem probable that this effect of light on luciferin has any connection with the luminescence of luciferin, but is one of the chance photochemical effects so common in organic compounds.

KINETICS

In the study of the kinetics of ordinary enzyme reactions the concentration of the substrate must be determined at definite intervals of time. Such a procedure is not possible with luciferin, as methods of analysis apart from the luminescence it gives are unknown. However, when luciferin and luciferase solutions are mixed, we observe a bright luminescence which represents light due to some initial concentration of luciferin. After mixing, the luminescence gradually decreases in intensity and a study of this decay curve, both as regards form and area involved, has given an interesting insight into the course of the oxida-

tion of luciferin. Quantitative studies on luminescence, therefore, involve measurements of luminescence intensity and total luminescence. These have been carried out by photographic, photoelectric, and direct photometric methods.

Amberson¹¹ first obtained decay curves by a photographic method and found them to be logarithmic and to give straight lines over a considerable temperature (5° - 35° C) range, if log intensity of luminescence is plotted against time. Since this decay curve is also the same as would be followed by a monomolecular reaction when reaction velocity, dx/dt , is plotted against time, the conclusion is drawn that intensity of luminescence (within certain limits) is determined by reaction velocity and that only one molecule of luciferin is undergoing transformation. Plotting log concentration of luciferin against time should also give a straight line for a monomolecular reaction but the experiments in which luciferase concentration or temperature is varied show that luminescence intensity is determined by reaction velocity, dx/dt , and not by concentration of luciferin at any given time.

The principal facts established by Amberson's work are:—

1. Stirring does not effect the luminescence intensity or the form of the decay curve.
2. The decay curve slope, i.e. the velocity constant, is approximately proportional to luciferase concentration.
3. Logarithmic plottings with two different concentrations of luciferin (made by allowing the luciferin to spontaneously oxidize to different degrees), but with the same luciferase, are parallel. The velocity constant is not affected, but light intensity (reaction velocity) is naturally less with smaller concentrations of luciferin. The velocity constants vary if the luciferin solutions are changed in concentration by dilution with water. Later work¹² has explained this discrepancy.
4. There is an initial flash of light when the luciferin and luciferase are mixed, too high to agree with the remainder of the curve. Its meaning is unknown.
5. The temperature coefficient is high. Amberson has collected the velocity constant values obtained in nine experiments and finds Q_{10} for different 10° temperature intervals to average 2.74. There is a tendency for the higher (25° - 35°) intervals to have a greater Q_{10} than the lower (5° - 15°).

⁹ Harvey, E. N., *J. Gen. Physiol.* **7**, 133, 1925; *Am. J. Physiol.* **77**, 555, 1926.

¹⁰ Harvey, E. N., *J. Gen. Physiol.* **7**, 679, 1935; **10**, 103, 1926.

¹¹ Amberson, W. R., *J. Gen. Physiol.* **4**, 517-558, 1922.

¹² Harvey, E. N., and Snell, P. A., *Proc. Am. Phil. Soc.* **69**, 303, 1930; *J. Gen. Physiol.* **14**, 529, 1931.

Harvey and Snell¹² made an extensive study of the sudden flashes, with rapid decay of luminescence, when luciferin solutions are mixed with fairly concentrated luciferase solution. A photoelectric cell, amplification and string galvanometer recording were used. Such luminescences may diminish to half intensity in 0.5 to 1.0 second. For these rapid flashes the following facts appear:—

(1) The decay is logarithmic if ratio of luciferin to luciferase is small; logarithmic plus an initial flash, if ratio of luciferin to luciferase is greater than five. The logarithmic plot of luminescence intensity against time is concave to time axis if ratio of luciferin to luciferase is very large.

(2) The velocity constant of rapid flashes of luminescence is approximately proportional to enzyme concentration, is independent of luciferin concentration, and varies approximately inversely as the square root of the *total luciferin* (luciferin + oxyluciferin) concentration. For large total luciferin concentrations, the velocity constant is almost independent of the *total luciferin*.

The distinction between *total luciferin* and luciferin is as follows. A luciferin solution (A) freshly prepared from *Cypridina* powder will contain a little oxyluciferin, due to unavoidable spontaneous oxidation. If allowed to stand 10 min. (B) much more oxyluciferin will form but the *total luciferin* (oxyluciferin + luciferin) will be the same. Luminescence records from A and B, on adding luciferase, will give the same velocity constants, but the light intensities from B will be much less. If, on the other hand, A is diluted with water (C), both luciferin and oxyluciferin will be diluted, and the velocity constant of C will be greater than A, and its luminescence intensities less. The *total luciferin* (oxyluciferin + luciferin) is less in C because of dilution with water. (3) The variation of velocity constant with *total luciferin* concentration (luciferin + oxyluciferin) and its independence of luciferin concentration are explained by assuming that light intensity is a measure of the luciferin molecules which become activated to oxidize (accompanied with luminescence) by adsorption on luciferase. The adsorption equilibrium is the same for luciferin and oxyluciferin and determines the velocity constant.

Stevens¹³ and also Anderson¹⁴ have undertaken a study of the total amount of light emitted by luciferin under various conditions, for the purpose of developing a simple quantitative method of determining luciferin and luciferase. In

Anderson's experiments the total luminescence is detected by a photocell, and the photoelectric current charges a condenser whose voltage is read on a potentiometer, using the Lindemann electrometer as a null instrument. By measuring rate of change of condenser voltage on the potentiometer the device can also be used for determining the velocity constant of the reaction. The results are as follows:—

(1) The total light emitted under uniform conditions is approximately proportional to the amount of luciferin initially present, and independent of the concentration of luciferase. Velocity constants are proportional to luciferase concentration for a standard luciferin concentration.

(2) From 18° to 28° C the total light emitted per unit of luciferin decreases from 2 to 3.5% per degree increase in temperature.

(3) The total light is less at pH = 7.8 than at pH = 6.8 and less at pH = 6.8 than at pH = 6. Less total light is emitted in m/60 than m/15 phosphate buffer at the same pH, and less light from m/20 phthalate buffer than from m/20 phosphate buffer at pH = 6.

More total light is emitted in presence of 0.34 m NaCl than in 0.02 m NaCl.

Although solvent conditions modify the luminescence values, under definite standard conditions of temperature, pH and salt content of medium, total light may be used as a quantitative measure of luciferin, and the velocity constant as a quantitative measure of luciferase concentration.

Studies of luminescence in heavy water show that in 81% deuterium oxide about 19% more total light is produced, and the velocity constant at half completion is reduced to about 60% of that in ordinary water.¹⁵

DISCUSSION

Dr. Giese: It is interesting to note that in this paper Harvey points out that the energy of reaction of only 1 of 100 molecules of luciferin is emitted as light. The energy given off by the other 99 must then be given off as heat. Yet Harvey was unable to detect a change in temperature in a reacting luciferin-luciferase system using the most delicate methods available. The total heat produced is thus very small in quantity.

Dr. Winter: Since there are both luciferin and luciferase present in the extract, why is the luciferin not oxidized?

Dr. Korr: In the absence of water the oxidation (i.e. the hydrogen transfer) can take place only very slowly, if at all. If atomic hydrogen

¹² Stevens, K. P., *J. Gen. Physiol.* **10**, 859, 1927.

¹⁴ Anderson, R. S., *J. Cell. Comp. Physiol.* **3**, 45, 1933.

¹⁵ Anderson, R. S., and Harvey, E. N., *J. Cell. Comp. Physiol.* **5**, 249, 1934.

is used, however, oxyluciferin will take it up (become reduced) even in the dry form. One would like to know if the luciferin-luciferase system is a peculiar one among chemiluminescent substances, in that molecular oxygen is absolutely required as a specific hydrogen acceptor, irreplaceable by any other. Harvey has made many attempts at such replacements with both biological and inorganic oxidising systems, but although oxidation of luciferin sometimes occurred, light was never obtained in the absence of oxygen.

Dr. Meyer: Is it known whether an organic peroxide is produced in the oxidation of luciferin?

Dr. Korr: Harvey has never obtained any indication of the formation of any kind of peroxide, using the usual tests with titanium chloride, and with catalase and peroxidase extracts.

Dr. Meyer: Catalase, to my knowledge, will act only upon H_2O_2 and not upon organic peroxides; the same holds true for peroxidase; but in aqueous systems an organic peracid will very readily, in most instances, be hydrolysed to H_2O_2 . In some instances, e.g., plithal-maroperoacid, the liberation of iodine from KI is very slow and one has to allow sufficient time for the test.

Dr. Walsl: What is known of the chemical nature of luciferin and luciferase?

Dr. Giese: Harvey has shown that the luciferase of *Cypridina* has the properties of a protein, while luciferin (and oxyluciferin) of the same animal has many of the properties of a proteose. Neither has been obtained chemically pure, but R. S. Anderson, working in Harvey's laboratory, has made some progress in this direction with luciferin.

Dr. Harris: The fact that the efficiency of this system is known suggests the possibility of

using it for quantitative determination of small amounts of oxygen present in unknown systems, as luminescent bacteria are already used.

Dr. Giese: I think Harvey implies simply an order of magnitude in his statement of the efficiency of the luminescent system of *Cypridina*, since his corrections, for other oxidation occurring at the same time, are very rough. But as a qualitative indicator of minute quantities of oxygen, luminescent bacteria are indeed excellent and have been used for a long time. Molisch showed that in a mixture of algae and luminescent bacteria in which the oxygen supply had been exhausted and in which luminescence had ceased, a strong luminescence could be produced by merely exposing the mixture to even so weak a source of light as a match for a fraction of a second.

Dr. Korr: Harvey has also made an estimate of the efficiency of bacterial luminescence. Using the method of indirect calorimetry and determinations of intensity and quantity of luminescence on the assumption that the uptake of one molecule of oxygen in the oxidation of luciferin gives rise to one quantum of light he estimated the efficiency to be about 26%, 640 lumens per watt being 100%. The bacterial-luminescence problem, however, is a much more difficult one since all attempts to obtain luminous extracts have proved quite fruitless; the capacity for luminescence seems to be intimately associated with cellular structure. This is less surprising, perhaps, if one thinks of luciferase as a specialised type of dehydrogenase, and of luciferin as its substrate. The bacterial dehydrogenases are known to be closely bound up with cell structure (the cell surface, according to Quastel), active extracts of such enzymes from bacteria being very rare, but relatively common from other cells and tissues.

BASIS OF RADIATION MEASUREMENTS

F. S. BRACKETT

In medicine, just as one often finds in other fields of practical emergency, the application of physical and physical chemical methods has far outreached the understanding of the mechanism by which such methods prove effective. While our knowledge of physical and chemical mechanisms which are brought into play by the absorption of radiation is very imperfect, certain broad considerations lend definite shape to our picture of these phenomena. Since water forms the main constituent of all biological systems, its absorption characteristics mark out the regions of chief biological interest. In the tremendous sweep of the known electromagnetic spectrum, extending from the cosmic rays of less than $1/1000$ of an Ångström wavelength, wherein almost a trillion waves can be crowded into the short distance of one centimeter, to the region of long electromagnetic waves, in excess of 1000 kilometers, or 100 million cm., for a single wavelength, we find that by far the largest part of the electromagnetic spectrum is totally absorbed in passing through $1/10$ mm. of water. From the standpoint of biological interest, therefore, a large part of the electromagnetic spectrum may be thought of as being blotted out by the opacity of water, and hence by the first thin superficial skin layers. Our interest then centers upon the recurrent regions of relative transparency. First we have the gamma ray and X-ray region, bounded on the long wavelength side by the Grenze rays; then another region extending from 1750 Å or 175 m μ in the ultraviolet to about 2.5 μ in the infrared; and another region sometimes termed Hertzian wave, lying between the deep infrared and the range of radio broadcast, the region of diathermy. For the purposes of this discussion we shall be concerned only with the region of transparency including and surrounding the visible range of wavelengths.

Probably the most striking characteristic of this region is the great selectivity which one encounters. Absorption often mounts by a factor of over 100 times in the brief range of only 20 m μ . Many different types of mechanism may be set in motion by the absorption of radiant energy. In the shore interval of but one log unit from 0.2 to 2.0 μ we pass from a quantum corresponding to more than 140 k. cal. per mol down to 14 k. cal. per mol; in other words, from energy in excess of most of our familiar chemical reactions down to a range of common thermal disturbance. It is natural, then, that the short wavelength high energy region should lead to many and violent chemical changes, whereas the near infrared be-

comes equivalent to relatively small temperature changes with little effect upon chemical reaction. Evidently then the ultraviolet presents a powerful agency for producing changes. The difficulty is that the changes may be of too great variety. The great interest which attaches to the visible and very near ultraviolet is that here we find more specific and more restricted chemical reactions. From these considerations, it becomes evident that the distinction between different wavelengths is of the utmost importance. Curiously enough, little is known as to the relative wavelength effectiveness of such physiological effects as have received some degree of recognition.

All too commonly the whole ultraviolet is lumped together as though it offered little possibility of differentiation in possible results. Yet in the brief interval from 310 to 290 m μ , we pass from wavelengths where few cells suffer lethal effects to the point where practically all cells can be killed by radiation. Different cells exhibit marked differences in the threshold or longest wavelength of lethal action, and tremendous differences above those wavelengths in the rate of lethal action. For instance, in Fig. 1, curves 1 to 3 show the lethal effect for algae (*Chlorella vulgaris*)⁽¹⁾; curves a,b,c, and d for bacteria (*Staphylococcus aureus*), from data by Gates.⁽²⁾ However, it should be pointed out that while these are in each case curves of equal dosage plotted with percentage killing against wavelength, corresponding curves represent two orders of magnitude less energy for bacteria than for algae. Because of the great difference in relative lethal action, it has been possible to free algae from bacteria where presumably the two present symbiotic relationship.

On the other hand, compare these curves with curve e, an average curve prepared by Coblenz⁽³⁾ for the relative erythematous effect of equal energy. As a further evidence of selectivity and differentiation, note the marked difference in character of this curve from the relative lethal effect on simpler organisms.

Mercury arcs and high temperature carbon arcs without filtration are in every day use. The results of treatment with such sources are commonly referred to simply as ultraviolet treatment. Yet the mercury arc not only radiates at various wavelengths from 225 to 313 m μ , but also presents a very strong line at 365 m μ , others at 405, 436, 546, 578 m μ , and a strong line under certain conditions of excitation at 1.1 μ . The carbon arc, on the other hand, radiates with increasing intensity as we pass from the ultraviolet through the

visible, reaching a maximum between 700 and 800 $m\mu$, thereafter decreasing very slowly toward longer wavelengths. While the relative spectral emission in the blue and ultraviolet may be increased markedly by the introduction of salts into the core of the carbon, by all odds its greatest radiation occurs in the near infrared, and the visible far outweighs the near ultraviolet. Under these circumstances, conclusions as to the cause of observed effects, without some effort at determining the energy absorbed and effective may certainly be misleading in the extreme.

In order to further clarify the requirements which we must place upon radiation measurements, it is necessary to bear in mind certain of the simpler considerations of absorption mechanism. For a wavelength corresponding to a frequency ν , the molecular system changes in energy content by an amount $E_2 - E_1 = h\nu$, where h is Planck's constant. The energy absorbed may affect the molecular system in a variety of ways. Small amounts of energy may be thought of as simply changing the speed of rotation of the molecule as a whole. Wavelengths producing this type of energy change lie in the deep infrared outside the region of immediate concern. From 0.9 to 2.5 μ , most of the energy absorbed goes into changing the vibrational state of the molecule, namely modifying the vibrations of the atoms with respect to each other. Accompanying such vibrational changes are rotational changes as well. The latter, however, involve relatively small energy differences. A curious observation of great importance is that such absorption seldom, if ever, leads to the dissociation of the molecule. Energy present in vibration within liquid or heterogeneous phase apparently quickly degenerates into intermolecular motion, thus resulting in a rise in temperature. For this reason we are probably justified in regarding the infrared beyond roughly 900 $m\mu$ as producing simply thermal changes. It is evident from this standpoint that infrared therapy should be regarded as a form of thermal therapy.

The region from the visible to 1.4 μ and to some extent as far as 2.5 μ is of particular interest because of the fact that here we find the greatest transparency within the general range under discussion. Energy can be detected through as much as 5 mm. of flesh. Since the fall in energy is roughly exponential, most of the energy is, however, absorbed in the first mm. According to the observations of Bachem and Reed, ⁽⁴⁾ some 60 to 70% of radiation between 750 and 900 $m\mu$ are transmitted through the epidermal layers. Approximately 50% of the incident radiation is absorbed in the corium, from 17 to 20% reaching the sub-cutaneous tissue. The observations of Cartwright ⁽⁵⁾ place the maximum transmission be-

tween 1.0 and 1.2 μ , though he reports considerably larger percentage of transmission. Recent work of Hardy (unpublished) supports the smaller values observed by Bachem and Reed, but places the maximum of transmission more nearly in agreement with Cartwright. Exact evaluation of the transmission characteristics of the different cutaneous and sub-cutaneous layers is complicated by the large degree of scattering of radiation which is encountered. Maximum penetration is determined by three considerations: the absorption of water, the absorption of tissue, and the scattering.

Without going further into the nature of the absorption in this region, certain general conclusions can be drawn. The ordinary infrared therapeutic lamp which appears dull red has its maximum in the region from 4 to 5 μ , where such radiation is absorbed in the superficial layers and can hardly conceivably be different from other types of superficial thermal treatment. If penetration has a definite therapeutic value, one should use the high temperature tungsten lamps or carbon arcs, filtered through from 1 to 5 cm. of water, in order to remove the surface absorbed component of radiation. Or better yet, if one isolated the 1.1 μ mercury line, one would then have the ideal source of penetrating radiation. Using unfiltered solar radiation, Sonne ⁽⁶⁾ has reported temperature increases of some 4 degrees above the surface temperature, at 5 mm. below the surface. Laurens and his co-workers have failed to support the large temperature increases he has observed at greater depths. If radiation in the region of maximum transmission were used and the surface temperature maintained by water cooling, greater temperature differentials could be obtained than those possible with unfiltered radiation.

In the visible and near ultraviolet, absorption of radiant energy produces chiefly a change in electronic configuration. This may be accompanied by smaller energy changes due to vibration and rotation. As a result of such change in electron configuration,

- 1) no external change may take place, the electron configuration returning to normal with emission of energy (fluorescence), or
- 2) dissociation may occur, leaving the components either
 - a) in their normal or least energetic electron configuration, or
 - b) one or more of the components may be left in a higher electron configuration, thus being capable of special function, or
- 3) the energy may be lost by interaction with other atoms or molecules, which is termed second class collision.

It may be desirable to examine a little further

the effects which ensue from these primary changes.

DISSOCIATION

While dissociation may conceivably result in the production of ions, there is little evidence of such occurrences in this region. However, it must be borne in mind that most evidence in regard to mechanism is drawn from gas phase observations, and the conditions in liquid and heterogeneous phases are much more favorable to ionic dissociation. A mass of evidence exists indicating the common occurrence of dissociation into atoms and free radicals. These set in motion many types of chemical reactions which would otherwise require large amounts of energy, or on the other hand, where the process is exothermal, may produce long chain reactions. The types of reactions observed depend upon both the absorbing molecule and its surroundings. Hence the great possibility of both specificity and variety.

SENSITIZATION

The alternative mechanism, that of transfer of energy without chemical reaction, while having been clearly established in gas phase, has not been definitely shown in liquid and heterogeneous phases. However, since there has been little conclusive work in this field it remains a very interesting possibility. The sensitization of photographic plates, photosynthesis, cases of specific sensitivity such as hydroa have often been attributed to this mechanism. In other words, a large variety of photobiological effects in which the concentration of absorber remains more or less stationary have been attributed to sensitization or second class collision. Such conclusions are, however, very uncertain, since many mechanisms for cyclic regeneration of the original material can readily be proposed.

If the quantum of energy is sufficiently great, undoubtedly the electron is removed from the molecule. This may occur either with or without immediate dissociation. From experience with better known gas phase phenomena, electron escape, while certainly occurring in the X-ray region, would not be expected to occur in the visible and near ultraviolet. However, the effect of neighboring molecules which are inevitably present in liquid, heterogeneous and solid phases may readily make electronic escape possible and probable. Much further work on even primary mechanisms is required in this field.

With these postulated mechanisms in mind, what conclusions can we draw regarding wavelength specificity or selectivity?

1. A molecule associated with the immediate system affected must present absorption to radia-

tion. Since absorption bands in the visible and near ultraviolet may be narrow and since the relative absorptive powers may mount by many logarithmic units, the difference in effect of one wavelength from another is likely to be enormous. This point cannot be too greatly emphasized. Since sources in common use, such as the mercury arc, may be thought of as radiating to all intents and purposes at certain definite wavelengths and practically not at all at other intervening wavelengths, the wavelengths at which a line occurs determines whether it produces a great effect or none at all.

2. Absorption may take place but the energy of a single quantum may be insufficient to bring about the reaction. As a result the absorbed energy may be reemitted in fluorescence or degenerate into heat with no result. In such cases, one is dealing with a threshold or longest wavelength for which the photochemical action may take place. In gas phase, these occur sharply and are often readily established. In a biological system, however, due to the effects of neighboring molecules these are likely to extend over ten or more $m\mu$. In cases where no absorption occurs in the region of the threshold, one can gain little definite information about the threshold. However, if the threshold occurs within a band, one finds that for shorter wavelengths a photochemical reaction takes place, while for the longer wavelength side of the band no reaction ensues.

3. Still other considerations may modify the effectiveness of absorbed energy. Competing reactions may interfere with the end effect observed. Possibility of degradation of energy without producing the particular effect may lead to low efficiency. Altogether then many mechanisms interfere with the end result. Consequently the efficiency with which absorbed energy contributes to a possible reaction may vary over a wide range.

Three things then are essential to our knowledge of a photochemical mechanism: 1. absorptive characteristics, 2. threshold or quantum sufficiency, 3. quantum efficiency. These three things determine the overall sensitivity or response to equal energy incident at different wavelengths. In all this, we are assuming that the energy is actually incident upon the absorbing molecule. The intervention of screening material such as inert pigment or tissue will of course modify the apparent result. For instance, the minimum which we note in Fig. 1 for erythema effect, occurring at 280 $m\mu$, occurs in a region of enhanced absorption, according to Bachem and Reed. Thus at least a part of the fall in erythema effectiveness in this region may be attributed to screening.

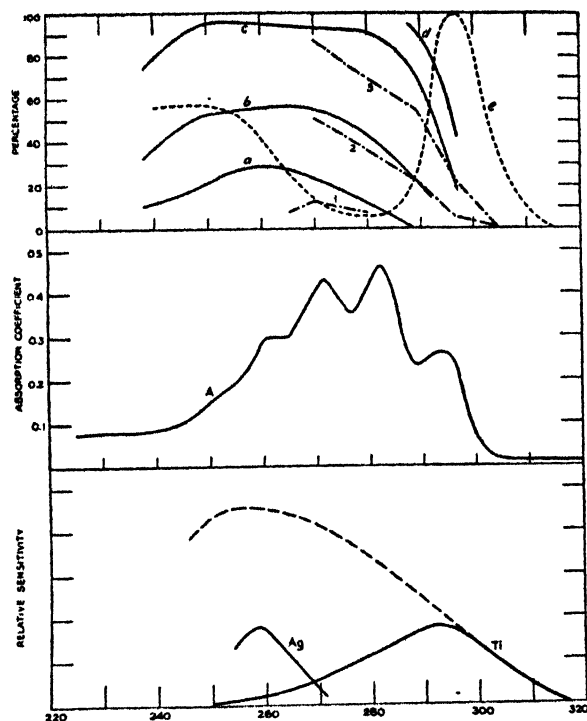


FIGURE 1

Upper section. Full lines curves—lethal effective-ness *Staphylococcus aureus* (Gates). Dosage: a) 50 ergs/mm.² b) 100 ergs/mm.² c) 250 ergs/mm.² d) 500 ergs/mm.². Dash-dot curves—relative lethal effect for alga *Chlorella vulgaris* for different dosages (Meier). Dotted curve e—erythema effective-ness (Coblentz).

Middle section. Absorption coefficient of unirradiated ergosterol, concentration 80 milligrams per liter (Reerink & van Wijk).

Lower section. Relative sensitivity of silver and titanium photocells. Full line curves in corex D. Dotted curves in thin corex A.

As an illustration of these points, let us consider certain restricted aspects of the transformation of ergosterol into vitamin D. Curve A, middle section, gives the absorption coefficients determined by Reerink and van Wijk.⁽⁷⁾ Presumably the energy initiating the first step in the transformation of ergosterol into vitamin D is absorbed somewhere in this region. The absence of absorption at longer wavelengths, would be sufficient to prevent any effect taking place at longer wavelengths, regardless of the photochemical threshold. If the quantum energy required to produce the reaction is small compared to that available in this region (over 95 k. cal. per mol) it is readily possible that the introduction of a sensitizer providing the necessary absorptive character may enable this reaction to proceed even in the visible. Or on the other hand, a molecule absorbing the visible might through

dissociation initiate a somewhat similar reaction or series of reactions resulting in biologically potent material. Evidence bearing on this matter will be presented later in the symposium by Dr. Karl Meyer.

Furthermore, it does not follow that the transformation of ergosterol into vitamin D or even the first step in this transformation will necessarily follow the absorption curve. Recent evidence has indicated that a number of intermediates occur before the formation of biologically active products. Intermediates luminesterol and tachysterol are both reported to have major absorption in the region of 280 mμ, and in the vicinity of 265 mμ, the latter also showing a band at 294 mμ, while a strong band is attributed to calciferol or one form of vitamin D at 265 mμ.⁽⁸⁾ Unquestionably over-irradiation leads to biologically inactive material. While it is beyond the scope of this discussion to attempt to adequately treat the evidence at hand in this rapidly moving field, let us consider some of the implications of the evidence cited. If luminesterol and tachysterol represent way stations in the transformation of ergosterol into vitamin D and as has been contended, radiation is necessary for each successive step, the overall process should proceed with much greater efficiency in the region of 294 mμ, where luminesterol presumably represents relatively less absorption. It should be possible for the process to proceed and the product to be formed in the region of 265 mμ, inasmuch as both postulated intermediates present absorption. If now absorption by calciferol leads to the formation of biologically inactive material, the same wavelength, 265 mμ, which leads to its formation might also simultaneously lead to its destruction, so that the concentration of active product would depend upon a balance of rates.

On the basis of this premise, the overall efficiency for the transformation of ergosterol into active product should exhibit a wide variation. While the evidence at hand is entirely inadequate to arrive at any definite conclusions as to mechanism, it is evident that while absorption is a necessary condition for photochemical reaction, it tells relatively little concerning overall effectiveness.

In view of the considerations regarding efficiency which have just been discussed, the results obtained by J. W. M. Bunker from monochromatic treatment of albino rats, using mercury arc source, are of particular interest. This work was reported at the Conference on Spectroscopy at Massachusetts Institute of Technology on July 19, 1935. The energies required for minimal dose according to figures which I have received indirectly are given in the second column with corresponding wavelengths in the first.

Wavelength in A.	Ergs	Transmission of Corneum	Transmitted Energy	Absorption Coefficient
3654				
3022	750,000	35%	262,000	.05
2967	450,000	30%	135,000	.2
2804	570,000	15%	85,500	.4
2652	670,000	16%	107,000	.3
2536	720,000	18%	130,000	.18

The longest wavelength for antirachitic effect is greater than 3022 A. Greatest overall effectiveness appears to be in the region of 2967 A.

If we assume that the corneum acts as an inert screen, these values should be corrected for transmission. Lacking specific data for the specimens used, we might assume values taken from the curves by Bachem and Reed. We now obtain the least energy for minimal dose in the region of 2804 A, the longer wavelength side of the band proving on the whole less efficient than the shorter. However, we are still dealing with energy incident, rather than energy actually absorbed. Since no data are available for the absorption of ergosterol or any of its products *in vivo*, nothing can be said as to the absorbed energy. It will be noted, however, on comparison with the absorption coefficients obtained in solution, that the least transmitted energy is required in the region of greatest absorption coefficient, and that a roughly reciprocal relation exists for all other observed values. There is no evidence from these data of any sharp changes in efficiency within the band such as might have been expected from the absorption bands of intermediates and product. One might question whether the intermediates identified necessarily act as way stations, or rather possibly as simply alternative courses by which biologically active product is reached.

A further observation can be made which I believe is of considerable importance. Since approximately twenty times less erythema results in equal energy exposure at 280 as against 300 $m\mu$, it is immediately evident that far greater therapeutic effectiveness could be obtained for minimal erythema by irradiation at 280 $m\mu$ as against the usual practise of using sources rich in the region of erythema response. Magnesium offers a group of lines which are excellently placed for this purpose. No better illustration could be found for the fallacy of using the erythema response as a measure of therapeutic effectiveness. It should be further noted that the erythema response curve extends to somewhat greater wave-

lengths, approximately 10 $m\mu$, than does the absorption curve of ergosterol. While this interval may seem relatively small, it has considerable bearing on the effectiveness of solar radiation, which during a large part of the year does not extend in appreciable intensity to the region of effectiveness observed by Bunker. However, the longest wavelength of observable antirachitic effect will have to be determined at very high intensities in order to make any valid observation on the effectiveness of solar radiation, which rises so rapidly in intensity from its shortest perceptible wavelength.

Let us now turn to an examination of what means we have at our disposal for measuring, producing and isolating radiation. The black body, being one which absorbs all radiation falling upon it, and transforms such radiation into heat, is the inevitable starting point for the measurement of radiation. For the purpose of this discussion, we shall assume that the distribution of radiant power as a function of wavelength is known for any temperature of black body and that the temperature of the black body is readily determined by means of pyrometer or thermocouple. Furthermore, since all radiation incident upon a black body is totally absorbed regardless of wavelength, its thermal equilibrium provides an immediate measure of radiation independent of wavelength. Without going into the details regarding black body detectors, since thermocouples with blackened receivers will serve that purpose, we may regard such a means as always at our disposal. Since such a black body detector absorbs all wavelengths, it immediately gives us a measure of total radiation. However since most sources emit more radiation in the near infrared than they do in the visible, the infrared is likely to receive the most weight in total energy measurement. Since the percentage of total radiation which is emitted in the visible or ultraviolet is small, total radiation measurements are of no value in immediately determining the energy in a particular region unless the distribution of energy as a function of wavelength is accurately known. Furthermore, where filters are used to isolate particular parts of the spectrum, for instance red, yellow, green, or blue, it cannot be assumed that these necessarily remove the infrared. In the majority of cases, they do not. Red and yellow filters in general freely transmit the infrared. Some green filters remove it, while others do not. Most blue filters transmit a large part of the infrared energy. Since most sources emit most strongly in the infrared, thermocouple measurements on source plus filter are chiefly measures of infrared transmission.

It happens that we have a wide range of filters which transmit all longer wavelengths and absorb

all shorter wavelengths, with a reasonably sharp transition range. A fairly well-graded set of filters may be found with cut-offs occurring anywhere from 700 to 240 $m\mu$. Such filters offer a number of very interesting possibilities. If a continuous source emitting radiation at all wavelengths is combined with a short wave cut-off filter, a series of exposures may be made with shorter and shorter short wave limits. By this means, the longest wavelength for which a photochemical reaction will occur may be roughly determined.

Coblentz⁽⁸⁾ has proposed an ingenious method by which a thermocouple may be used to determine the radiation in a limited range. A thermocouple is constructed with two opposing junctions which if equally illuminated yield no deflection. If two filters are chosen which differ only in short wave cut-off, one may be placed over each couple. The resulting sensitivity arises from the difference in transmission of the two filters. Thus, in Fig. 2, in the top section, let us consider the transmission curves of barium flint (BF) and corex D (CD). It is assumed that for all wavelengths longer than 400 $m\mu$ the two transmission curves coincide. Thus the balanced thermocouple method with these two filters yields a sensitivity curve indicated CD-BF in the middle graph. If quartz is combined with barium flint, one obtains the sensitivity curve Q-BF. This latter curve has been proposed by Dr. Coblentz as a standard means of measuring ultraviolet radiation. It has the great advantage of being non-selective from 260 to 320 $m\mu$. Since most of the important lines of mercury in the ultraviolet occur between 254 and 313 $m\mu$, all in a range of practically constant sensitivity, and then very little radiation until 365 $m\mu$, such a detector furnishes an excellent method for evaluating the energy short of 330 $m\mu$ in a mercury arc. However, if some source were to be used which exhibited strong radiation at longer wavelengths than 320 $m\mu$, such a detector would lead us into great error if we were to make direct comparisons with the mercury arc.

Let us now consider such a detector as a means of evaluating the erythral effectiveness of a source. For this purpose, the erythral effectiveness curve has been plotted on the same graph, dotted line E. Evidently, if the standard balanced thermocouple method were used to compare a magnesium arc having a strong line in the region of 280 $m\mu$ with the mercury arc having strong lines at 297 and 302 $m\mu$, the results would not be at all in proportion to erythral effectiveness. In order that the results may be fairly interpreted it is necessary to have a complete knowledge of the relative energy radiated at each wavelength.

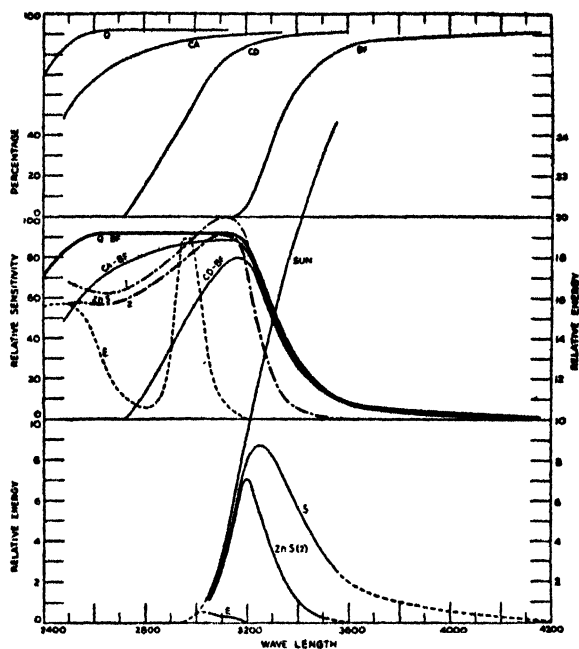


FIGURE 2

Upper section—transmission of filters.

BF—barium flint 3.18 mm. thickness
 CD—corex D 2.3 mm. "
 CA—corex A 2.86 mm. "
 Q—fused quartz 4.7 mm. "

Center section—sensitivity of detectors and erythral response curve.

Q—BF Balanced thermocouple with quartz and barium flint filters

CA—BF Balanced thermocouple with corex A and barium flint filters

CD—BF Balanced thermocouple with corex D and barium flint filters

E Erythema

ZnS-1 Lithopone A (Brickwedde)

ZnS-2 Balanced zinc sulphide method with quartz and barium flint filters.

Lower section—response to solar energy.

Sun Solar energy (Brian O'Brien)

S Response of standard balanced thermocouple to solar energy

ZnS-2 Response of balanced zinc sulphide detector to solar energy

E Erythral response to solar energy

Another method which has had fairly wide use is the zinc sulphide method developed by Janet Howell Clark. This has the advantage of escaping the necessity of using electrical instruments. Its sensitivity of response is indicated by Curve 1. If comparative darkening is observed with and without a barium flint filter, a sensitivity curve of the type ZnS-2 may be obtained. Evidently, such a response curve fits more closely the erythral curve than does the balanced thermocouple method.

In order to illustrate the difficulty into which one may be led unless accurate corrections can be made from knowledge of radiation distribution, let us attempt to evaluate the solar energy effective in erythema. To this end, the solar energy curve under certain particular conditions has been plotted from data by Forsythe,⁽¹⁰⁾ relative energy being indicated at the right and wavelength at the bottom. The response by means of the balanced thermocouple is indicated by S in the lower graph. The response of the zinc sulphide method ZnS-2 is also plotted and finally the energy actually effective in erythema by full line curve e. It is immediately evident that both methods of detection respond to an enormously greater range of wavelengths than does erythema. If the short wavelength limit of solar radiation were to shift to $310\text{ m}\mu$, relatively little erythema effect would remain, whereas both detector methods would still show a large response, though the zinc sulphide method would follow more closely the relative erythema effectiveness. While of course most of the work in which such methods have been utilized has been with full knowledge of these difficulties and corrections made as far as possible, obviously unless the solar distribution curve were accurately known under each set of conditions, relative evaluations might be in error by a considerable magnitude.

From this type of discussion, one realizes the importance of having detectors which follow closely the response curve of the phenomena to be measured. In other words, one desires a type of weighted energy measurement if one really wishes to evaluate the relative effectiveness of different sources or of the same source under different conditions. Furthermore, the use of such a weighing curve requires a complete knowledge of the overall effect, wavelength by wavelength, for equal energy. This can only be done by means of a monochromator or some apparatus which isolates a narrow wavelength band. Then the photochemical response can be determined for each wavelength range and compared with the energy as determined by means of a thermocouple. Another difficulty arises from the fact that the relative energy required at each wavelength may differ markedly for different degrees of the same effect. This is particularly so in the case of erythema, where the relative sensitivity in the short and long wavelength maxima reverses itself for high erythema as compared to low. Thus, really critical agreement could be obtained only for one particular degree of effect.

Two radiation phenomena in connection with physiology, which have received especial attention, are erythema and vision. Such relative sensitivity curves may be used as weighting curves in the valuation of energy effectiveness and a

system of units may be established for measuring effectiveness. On this basis, for vision the whole illuminating system has been built, and on a similar basis an erythema system has been established by the Council of Physical Therapy. Without at the moment going into further detail regarding the steps by which such a perfectly definite system of measurement can be established, let us emphasize one point. Such a system is absolutely good for nothing else than for measuring visual effectiveness or erythema effectiveness as the case may be. Yet continually these two systems are being used for other purposes. Photocells having response curves not in the least like the visibility curve are customarily specified in terms

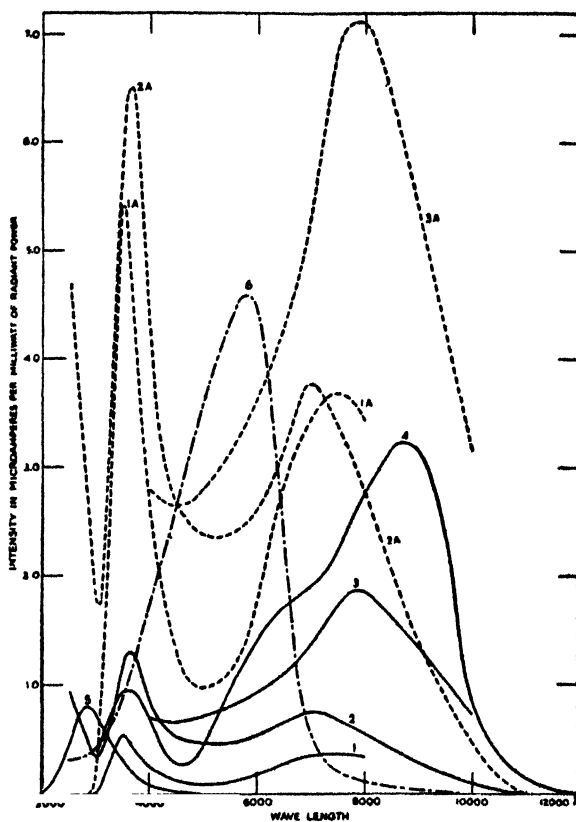


FIGURE 3

Relative sensitivity curves of commercial photo-cells.

1. PJ-22 vacuum; G. E. (manufacturer), 0.9 sq. in. (area).
- 1A. PJ-23 gas-filled; G. E.; 0.9 sq. in.
2. Visitron type AV, quartz envelope, vacuum; G. M.; 1.1 sq. in.
- 2A. Visitron type A, quartz envelope, gas-filled; G. M.; 1.1 sq. in.
3. Western Electric, vacuum; Western Electric.
- 3A. Western Electric, gas-filled; Western Electric.
4. FJ-114; G. E.; 0.9 sq. in.
5. FJ-76; G. E.; 2.1 sq. in.
6. Weston Photronic cell; Weston Electric; 1.7 sq. in.

of lumens. Evidently the sensitivity in lumens will vary for every different type of radiation distribution. Without specifying the source of radiation used in calibration, sensitivity of photocells in lumens is absolutely meaningless. This is well illustrated by the sensitivity curves of different photocells given in Fig. 3. Curves 1 to 4 show the different types of caesium on caesium oxide on silver photocells most widely used. The corresponding dotted curves are for gas-filled cells with the same type of surface. In view of the great sensitivity which these cells exhibit in the near infrared, it is evident that the ratio of visible to infrared radiation from the source will greatly modify the response for a given luminous equivalent. The results obtained are critically dependent on the temperature of the source. Only the photonic cell, No. 6, at all approximates the visible, and even it extends over a much wider range.

Equally meaningless is the specification of a biological reaction other than vision in terms of lumens or other units of the illuminating system. Furthermore, evaluation of therapeutic effective-

ness in terms of erythral response is equally misleading unless therapeutic effectiveness very closely follows erythral response. There is so far as I know absolutely no reason for assuming such a relationship. To measure therapeutic effectiveness by means of erythral response is to measure it wholly by its damaging effect. This can have no meaning unless the sole benefit of radiation therapy arises in some way from the apparent injury as shown by erythema. The work recently reported by Bunker definitely establishes the fallacy in existing procedure.

As soon as the response for a given phenomenon can be established by means of a monochromatic study, it is readily possible to devise instruments which fairly closely follow the response curve of the phenomenon. When a good fit is obtained between the detector, sensitivity curve and the observed phenomenon, such an instrument furnishes an accurate measure of dosage. Fig. 4 shows a variety of photocells which lend themselves in combination with filters to the detection of almost any region of the spectrum. The upper section shows the common alkali metals, while the two lower sections show sensitivities obtained by Rentschler from specially constructed photocells. The short wave cut-off can readily be modified by use of filters, or extended to shorter wavelengths if the photocell is constructed in quartz.

As an example of a special detector, let us consider the desirability of determining the penetrating component of radiation for thermal treatment. In Fig. 5, I have shown the transmission curve

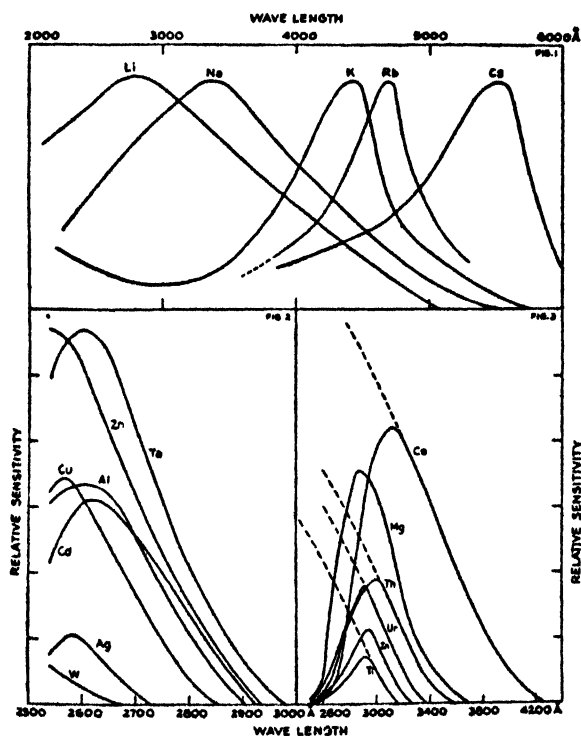


FIGURE 4

Relative sensitivity of special photocells.

1. Alkali metals (Hughes & Dubridge)
Li, Na, K, Rb, (Pohl and Pringsheim)
Cs (Campbell and Ritchie)
2. and 3. Westinghouse ultraviolet photoelectric cells (Rentschler). Full line curves in thin glass; dotted curves in quartz.

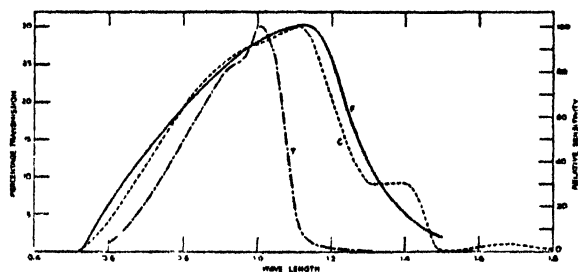


FIGURE 5

Flesh transmission and special detectors.
Full line curve F—transmission of 5.0 mm. thickness of flesh (Cartwright, Forsythe et al).
Dotted curve C—thermocouple with filters amethyst + heat resisting yellow shade yellow.
Dash-dot curve T—thalofide cell.

obtained by Cartwright⁽¹¹⁾ and corrected for surface reflection by Forsythe, and by means of the dotted curve C the response of a thermocouple with suitable filters which very closely approximate the transmission curve. Such a detector would very readily enable one to determine the

amount of penetrating radiation present in any source, provided of course that the transmission curve of flesh has been correctly evaluated. The sensitivity of a thalofide cell has also been plotted, which falls fairly well in the region. Of course the selection of radiation distribution on the basis of penetration will be determined by the depth at which one wishes to obtain the maximum energy absorbed. On the basis of Bachem and Reed's measurements, if one wishes to obtain the maximum absorbed energy in the malpighii, one would choose a distribution having a maximum in the region of $400\text{ m}\mu$.

As a further illustration of the possibility of fitting biological response curves, Fig. 6, upper

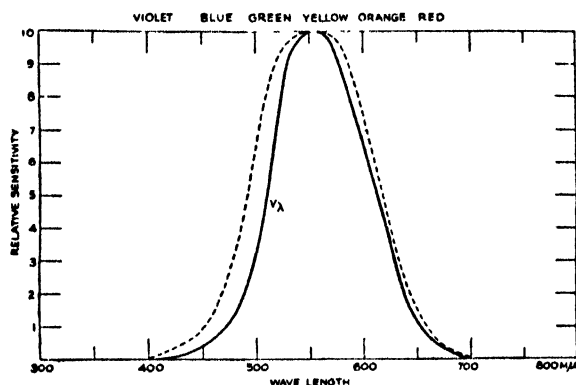


FIGURE 6

Visibility and special detectors.

V_λ —visibility.

Dotted curve—sensitivity of Weston photronic cell + heat resisting heat absorbing dark, 2.82 mm. thickness + Noviol 0, 16 mm. thickness (Corning filters).

section, shows the relative visibility curve obtained by combining the photronic cell with suitable filters. While the agreement is excellent, for the most part, it will be seen that if a selective emitter were used which presented a strong line in the region of $500\text{ m}\mu$, the response of the detector would be disproportionate to the visual response by a factor of some four times. Yet the full response of the photronic cell, which diverges widely from the visibility curve, is in every day use for photometric determination of all types of sources.

While such control instruments, approximating different types of biological response, are of great value in carrying out controlled experiments, their limitations should be clearly recognized. The distribution of energy of the source should be taken into consideration in determining the amount of error incurred. A second difficulty in establishing definite weighting curves and developing instruments whose response curves follow those

weighting curves is that early determination of the monochromatic response may be crude, and a whole system of units and measurements established on so unsatisfactory a basis that it will have to be modified, thus throwing all earlier data out of line. Such has repeatedly been the case in the illuminating system. A further danger is that such units will be used for other phenomena to which they do not apply.

Where one finds complicated response curves, it is often impossible to simulate those curves in a single detector. Turning again to Fig. 1, the combination of two photocells has been suggested, by which energy in the different parts of the ultraviolet can in a measure be determined. The titanium cell must be used with a suitable thickness of glass, in order to obtain the full line curve. If placed in corex A, one obtains the sensitivity indicated by the dotted curve.

In conclusion, let us emphasize that monochromatic determinations on radiation effectiveness are essential to any real progress in the radiation field, and that the establishment of special units of measurement are fraught with great danger and have been responsible for much of the chaotic condition which now exists.

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DISCUSSION

Dr. Blum: Brackett has indicated many important points to be kept in mind in the study of the effects of radiant energy on living systems, none of which can be too much emphasized. I am particularly interested in his pointing out that the use of illuminative system units, i.e. lumens, lamberts, etc. is confusing when applied to studies on organisms other than man. These units are based on the spectral sensitivity of the human eye and are not interpretable when applied to an organism having a spectral sensitivity different from that of the human eye, and are not inter-

pretable when applied to an organism having a different spectral sensitivity unless the characteristics of the source are specified, e.g. the color temperature of a tungsten lamp.

With regard to units, I should like to suggest that it might be more logical to express spectral sensitivity of organisms in terms of relative number of quanta rather than relative energies, since these sensitivities are related to photochemical reactions which are dependent upon number of quanta. The conversion from energy units to relative number of quanta is easily made, of course, by multiplying by the wavelength. The difference may not be significant, although it represents a considerable factor when comparing sensitivities over a wide wavelength range.

Dr. Brackett: I think that the latter point is well taken. We should really consider the number of quanta. Of course, if wavelength and energy are specified, it is sufficiently definite. So much confusion has arisen from the use of units which are completely indeterminate that I was inclined to emphasize that point especially.

Dr. Blum: I wonder if the intelligent use of filters will not be more practical than the use of a monochromator in many cases, because of the possibility of obtaining higher intensities.

Dr. Brackett: As to the relative merits of filters and monochromator for restricted wavelength bands, the transmission of a good monochromator generally exceeds that of a band pass filter. The transmission of a monochromator may be in excess of 40% so that for the solid angle which it can handle it is likely to be superior to "monochromatic filters" which commonly range from 3 to 35% transmission.

As I have mentioned there are excellent short wave cut-off filters which transmit in excess of 80%. Where the longest wavelength of effectiveness is in question they are especially useful or where it is desirable to remove known complicating short wave reactions. Unless one requires a very large solid angle of irradiation, the only disadvantage peculiar to the monochromator is its expense.

If one wants a wide band, the slits can be opened up and still give sharp edge cut-off. In one case the filter has particular advantage. When one wishes to build up the radiation density by cross-firing, it is readily possible with filters. Of course, more or less the same thing can be done by a battery of monochromators, provided the expense is not prohibitive. So far as expense is concerned, there is a wide range of experiments which can be done with a liquid prism. If the prism is placed with the refracting edge horizontal, stratification of layers with different temperature may be allowed to develop, the only result of

which is to change the apparent dispersive power of the prism. Furthermore, a large part of the energy incident on the prism may be cut out by preliminary filtration, thus minimizing the thermal effect on index of refraction. Such a monochromator may be built up with relatively little expense.

Dr. Mestre: In connection with the subject of low cost large aperture monochromators, there are several possibilities which should, I think, be mentioned. The first is the use of the simple and ingenious instrument designed by G. R. Harrison at the Massachusetts Institute of Technology and employed by Bunker in the rat radiation experiments mentioned by Brackett. This instrument has been fully described in the *Rev. Sci. Inst.* 5: 149-152, so that it will be only necessary to mention here that the dispersing system is composed of an 8 inch concave mirror of 96 inch radius immersed at an angle in a basin of distilled water. All of the important Hg arc lines are easily isolated, and working at f.6 in the range 4000-2500 Å, the energy output was equivalent to that obtained with a 3 inch quartz instrument working at f.3 and costing more than ten times as much. The use of ethyl cinnamate in hollow prisms should also be mentioned. The very low coefficient of temperature expansion of this substance greatly reduces difficulties arising from convection currents and its relatively low index of refraction permits the use of a prism angle of 65° instead of the conventional 60°, thereby materially increasing the dispersion. At some sacrifice of spectral purity, low cost and high intensities can be obtained by using the filament of a ribbon filament lamp as the entrance slit of the monochromator.

Dr. Brackett: Wherever it is feasible to use the sun as a source, one can dispense with the first slit and collimating lens, projecting a parallel beam upon the prism by means of the coelostat. Under these conditions, one obtains, no matter what focal length is used, a 500 Å spread, at a given point in the spectrum. In this way one can take advantage of the great intensities offered by the sun. I don't see any reason why one couldn't construct a prism 4 feet high with correspondingly large area of aperture. In this way it would be possible to grow a large number of organisms in a restricted wavelength band, thus obtaining really adequate statistical data. Of course one then has to use an integrating detector in order to obtain time intensity dosage, since solar radiation is subject to continual change of intensity.

Dr. Mayerson: Brackett's paper and suggestions have interested me very much. One of the chief reasons for the confusion in the field of radiation has been the failure of investigators to

accurately specify the quality and quantity of the energy which they were using. We should, perhaps, be a little lenient in our criticism, since the use of the only accurate methods available at the present time is out of question as far as the average worker is concerned, who lacks specialized apparatus and training. It seems to me that there is urgent need of (1) dependable sources, (2) simple and reasonably accurate methods of measuring and specifying their energy in different parts of the spectrum.

Dr. Harris: Taking an optimistic view, and assuming that apparatus of sufficient precision to make accurate measurements, of the kind Brac-

kett has mentioned, will become increasingly available in laboratories, I would like to point out the desirability of using as great care in respect to biological material as in respect to physical apparatus. Too frequently one uses fine physical apparatus for experimentation upon biological systems which are not well chosen. I would like to urge that when and as quantitative work is done, we take proper account of genetics and of other branches of physiology in the choice and use of the experimental material. I think that exceptionally good apparatus is most desirable. Exceptionally good animals and plants are also important.

PHOTOCHEMISTRY IN MEDICINE: A GENERAL OUTLINE

HENRY LAURENS

INTRODUCTION

The number of abnormal conditions primarily and specifically benefited by radiant energy, natural or artificial, is small in comparison with the number for which such claims have been made. Everyone now appreciates the fact that sunlight presents one of the benefits of an outdoor life, that it is one of the elements of climate that makes for physical and mental well being, that in extrapulmonary tuberculosis it is an auxiliary in promoting healing, that in rickets certain wavelengths are specific; but there is still manifest a tendency to exaggerate the vital importance of both natural and artificial sunshine and to make extravagant claims for it as a therapeutic force. Certain diseases and disabilities are partly due to deficient radiation, and doubtless the health of the community may be improved by providing more radiation where sunlight does not reach the small minimum required for health; but sunlight is only one of the many environmental factors that affect vitality. Climate in its relationship to health is not merely a question of radiation, but of radiation, fresh air, wind, temperature, altitude above sea level, humidity, etc. "Heliotherapy" includes all of these, as well as attention to diet and occupation. Radiant energy is of paramount importance to plants, but of secondary importance to animals. The fact that certain ultraviolet wavelengths activate vitamin D in the skin, and that exposure to sunlight promotes the healing of some tuberculous lesions does not imply that everyone is suffering from a lack of sunlight. Sunlight plays a subordinate part in the metabolism and vigor of normal man, who can get along with little of it provided his diet be adequate and that he take care of himself by getting plenty of fresh air and sleep.

For a general review concerning the nature of natural and of artificial radiant energy reference may be made to Laurens (1). Sunlight is our natural source of radiant energy for therapeutic purposes. In many places its intensity, however, varies too much, or is too weak for too great a proportion of the time, to permit of its being a practical source. In the choice and use of an artificial source there are two basic considerations: (1) the physical nature (quantity and quality) of the energy emitted by the lamp; (2) the specific part or parts of the spectrum of sunlight found most efficient in the particular condition it is desired to treat and which should thus be sought in sufficient quantity in the artificial radiation. As artificial sources, only the mercury vapor arc in quartz (low and high voltage types), the flaming

carbon arc burning cored carbons, and "heat" radiators, have been of practical importance. It has been abundantly demonstrated that the penetrating long waved luminous, and short waved infrared, rays emitted by tungsten lamps (200-500 watt) or electric heaters are of value in conditions requiring deep action. These rays have a definite place in therapy, being indicated when it is wished to improve the circulatory conditions at some distance below the surface, in order to furnish more nutritive materials and to remove waste. Such radiation takes the place, in all conditions where heat applicators are indicated (contusions, sprains, fractures, congestion, swollen and painful polyarthritis, neuritis, neuralgia, etc.), of the older fashioned plasters and hot water bottles, towels and baths, etc.

At the surface of the earth with the sun moderately high and a total intensity between 1 and 1.5 gm. cal. per sq. cm. per min. (70,000 and 105,000 microwatts per sq. cm.) the percentage of the ultraviolet is between 1 and 5, the luminous between 41 and 45, and the infrared between 52 and 60. When the sun is lower and the total intensity less, the ultraviolet is relatively decreased and the infrared increased. At high altitudes above sea level the total energy is increased, as is the percentage of ultraviolet, while the percentage of infrared is diminished.

The radiation from the flaming carbon arc is the closest approximation to natural sunshine. In one of our lamps with 25 amperes flowing through the arc (Eveready "Sunshine" carbons $\frac{3}{8} \times 6$ in.), the total energy emitted is 0.320 gm. cal. per sq. cm. per min. (22,400 microwatts) incident at a meter with a distribution of 6 p.c. ultraviolet, 50 p.c. luminous and 44 p.c. infrared. With a Correx D screen, which eliminates by absorption the shorter ultraviolet and the longer infrared rays not found in sunlight, the total energy emitted is 0.287 gm. cal. per sq. cm. per min. (20,090 microwatts), and its distribution 5 p.c. ultraviolet, 52 p.c. luminous and 43 p.c. infrared. When Eveready Therapeutic "C" carbons are burned in this lamp the total energy emitted has a value of 0.266 gm. cal. (18,620 microwatts), with a distribution of 9 p.c. ultraviolet, 24 p.c. luminous and 67 p.c. infrared. Compare the values for a Hanovia air cooled mercury lamp with a distribution (as given by the company) of 28 p.c. in the ultraviolet, 20 p.c. in the luminous and 52 p.c. in the infrared; and of an old Cooper Hewitt air-cooled quartz mercury lamp in my laboratory with a total energy output, when operated at between 4 and 5 amperes, of between 0.0562 and 0.0619 gm.

cal. per sq. cm. per min. incident at a meter, and with a distribution of 13 p.c. ultraviolet, 7 p.c. luminous and 80 p.c. infrared.

The amount of ultraviolet radiation shorter than $313\text{ m}\mu$ that can be applied to the body without producing a burn depends on the tolerance of the skin. This can be measured by the erythema produced, specifically a mild or "minimum perceptible erythema", or one that disappears in the course of 24 hours. The spectral erythemic reaction is produced only by ultraviolet rays of wavelengths shorter than about $315\text{ m}\mu$ with a maximum reaction at the wavelength $296.7\text{ m}\mu$ and a lesser maximum in the region of $250\text{ m}\mu$. The midday, midlatitude, sea level ultraviolet shorter than $315\text{ m}\mu$ in summer sunshine with an intensity of from 80 to 90 microwatts per sq. cm. produces a minimum perceptible erythema in

a unit based on the comparison between the erythema produced by measured amounts of heterogeneous ultraviolet radiation, evaluated by a balanced thermocouple and filter radiometer, in which the intensity (radiant flux) should not be less than the equivalent of 20 microwatts (200 ergs, 0.000285 gm. cal.) per sq. cm. of homogeneous radiation of the wavelength of maximum erythemogenic effectiveness, namely, $296.7\text{ m}\mu$.

SKIN

The skin reflects and transmits different parts of the spectrum quite differently, and different parts of the body behave differently; pigment, hair and blood being important elements in determining the relative amount of the energy absorbed. Figure 1 shows the relative penetration

LAYER	λ m μ	200	250	280	300	400	550	750	1000	(1400)	$\mu\mu$
		100	100	100	100	100	100	100	100	100	APPLIED
CORNEUM	.03	(100)	(81)	(85)	(66)	(20)	(13)	(22)	(29)	(56)	ABSORBED AND REFLECTED
		0	19	15	34	80	87	78	71	44	TRANSMITTED
+MALPIGHII	.05	(0)	(8)	(6)	(18)	(23)	(10)	(13)	(6)	(16)	ABSORBED
		0	11	9	16	57	77	65	65	28	TRANSMITTED
+CORIUM	2.0	(0)	(11)	(9)	(16)	(56)	(72)	(44)	(48)	(20)	ABSORBED
		0	0	0	0	1	5	21	17	8	TRANSMITTED
+SUBCUTAN.	25	(0)	(0)	(0)	(0)	(1)	(5)	(20)	(17)	(8)	ABSORBED
		0	0	0	0	0	0	1	0	0	TRANSMITTED
GENERAL REMARKS	EXTREME U-VIOLET	FAR ULTRA VIOLET				NEAR U-VIOLET		VISIBLE VIOLET GREEN RED		NEAR INFRARED	
	All absorbed by corneum. No radiation reaching germina- tium	Greatest absorption in stratum corneum. Some radiation reaches corium (papillae). No radiation reaches subcutaneous layers.				Relative- ly large absorption in stratum Malpighii.		Minimum absorp- tion in stratum corneum. Most radiation absorbed in corium.		Strongly increasing absorption in upper layers, decreasing in lower layers.	
						Pronounced radiation reaching subcutaneous layers				Practically no penetration	

FIGURE 1. Energy distribution in the layers of the skin. The number 100 designates the applied intensity. The encircled numbers represent percentages absorbed in each layer. The numbers in the narrow zones between layers represent the percentages of the original intensities transmitted through the layer above. (Bachem and Reed).

from 20 to 45 minutes, depending on the person. In winter the intensity is only about 20 microwatts (on the clearest days at noon) and the time is lengthened to from 3 to 5 hours. Forenoon and afternoon summer sunlight has an average intensity of 30 microwatts per sq. cm.

Some sort of standardization of measurement and of dosage is needed in order that results may be compared. The total energy should be ascertained by a non-selective method that can be calibrated in absolute units (thermopile and galvanometer), as must the erythemogenic radiation of wavelength $296.7\text{ m}\mu$ employed as a standard. Coblentz (2) has worked out a suggestion for

of important wavelengths between 200 and $1400\text{ m}\mu$ as determined by Bachem and Reed (3). Hasselbalch (4) reported a transmission of only 0.01 p.c. of wavelength $289\text{ m}\mu$ through $100\text{ m}\mu$ of skin, Bachem and Reed find it to be approximately 10 p.c.

The layer of capillaries in the epidermis absorbs the ultraviolet and a part of the visible rays at slight depths (red blood cells, of course, absorbing much more than the plasma). The absorption curve rises from the long waves to the short and there is a strong maximum between 415 and $400\text{ m}\mu$. The red cells which push through the capillaries absorb only 5 p.c. of the

wavelength $680\text{ m}\mu$, 92 p.c. of $405\text{ m}\mu$, and 72 p.c. of wavelength $290\text{ m}\mu$. The blood which flows through the skin is of great importance. The more the capillaries are filled, so much more the shorter rays will be taken up by the blood and given up to the organism as a whole, and so much more the long-waved energy will pass through the network of blood vessels and penetrate to the deeper layers of the body. Lucas (5) estimates that amounts varying from 55 p.c. for wavelength $313\text{ m}\mu$ to 26 p.c. for wavelength $294\text{ m}\mu$ may be transmitted to the dermal capillaries, and that human epidermis has from 1.5 to 30 times the transparency, stated by Hasselbalch (4) for radiation of wavelengths $404\text{ m}\mu$ to $289\text{ m}\mu$, respectively.

Sonne (6) does not believe that the beneficial action of irradiation is to be found exclusively in the ultraviolet region. During irradiation with the highest endurable intensity of visible energy he observed that the skin temperature rose to 43.8° ; with outer infrared to 45.5° C. (see Figure 2). Fifteen seconds after the irradiation the

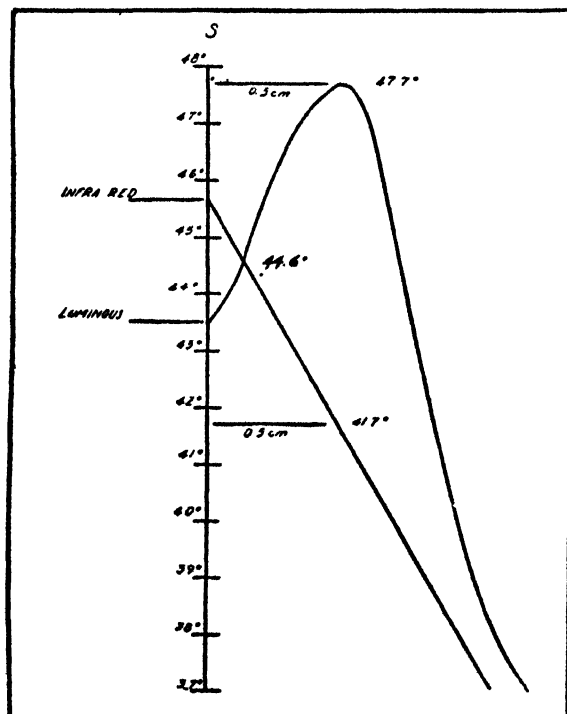


FIGURE 2. The heating of the skin and subcutaneous tissues during, and following, irradiation with the carbon arc. (Sonne).

relation was reversed, the skin temperature after irradiation with visible rays being 1° C. higher than after irradiation with infrared rays. According to Sonne the temperature of the skin during irradiation with outer infrared gradually

falls from 45.5° at the surface to 37° at 1 cm. depth; for visible rays the maximum temperature at a depth of 0.5 cm. is 47.7° C. ; during exposure to outer infrared the temperature at the same level is 41.7° C. At a depth of 0.12 cm. the temperatures are identical, namely, 44.6° C. The inner infrared wavelengths show a condition somewhat similar to that holding for the outer, except that they penetrate somewhat farther and thus the temperature slightly below the surface is somewhat higher than the surface temperature. Skin is very opaque to the long-waved infrared. These rays are absorbed in the outermost layers, and produce a tingling and painful sensation there, accompanied by a momentarily appearing and disappearing sensation of warmth, while the shorter infrared, as in sunlight, penetrates for 2 to 3 cm. and produces there quite a marked increase in temperature, as a matter of fact, above the highest fever temperatures ever recorded. Somewhat similar results were reported by Loewy and Dorno (7). Sonne believes this deep heating, by the luminous and shorter infrared portion of the spectrum, to be the main feature of the curative action of radiant energy. In work now being carried out in my laboratory on the essential function of skin pigment, we have not yet been able to substantiate these claims concerning penetration and heating.

ERYTHEMA AND PIGMENTATION

The almost immediate reddening of the skin after irradiation with energy containing ultraviolet, visible and infrared rays is due to radiant heat (infrared and luminous rays). This heat hyperemia, which frequently has a mottled appearance, is not restricted to the irradiated parts of the skin, and disappears soon after the irradiation. It is followed in a few hours by an erythema due to the action of the ultraviolet rays on the cutaneous capillaries. The usually diffuse and homogeneous redness of this erythema is confined strictly to the irradiated part, and, according to the intensity of the radiation, may be combined with blistering and hemorrhage. The inflammation lasts for some time, to be followed by peeling and pigmentation. The dermatitis, the end result of the aggregation of colloidal particles, is a pathological outcome of the skin's physiological reaction. Solar erythema and the dermatitis caused by carbon and other arcs are one and the same thing. Finsen (8) clearly demonstrated that the ultraviolet rays of the sun were powerfully active, while some activity could be detected in the violet and blue rays in producing erythema. In an experiment in which Finsen exposed his own arm, of which certain areas were protected by colored and clear glass plates, for 20 minutes

to the energy emitted by a 40,000 candle power arc lamp, the erythema began to appear in the uncovered parts after 3 hours, reached a maximum in 12 hours, decreased again after 2 days, and disappeared gradually in about 10 days, during which desquamation of the epidermis took place, followed by pigmentation. This pigmentation faded very slowly and could still be discerned after 5 months. After about 6 months the arm was uniformly white, but even then a definite after-effect upon the skin capillaries could be seen: when the arm was rubbed the skin became red, but the redness was less pronounced in the areas which had been covered during the experiment. The radiant energy had produced an increase in the excitability of the capillary wall toward mechanical stimuli.

Dorno believes that all erythema is followed by pigmentation, and that pigmentation never occurs without previous erythema. But two of the most experienced heliotherapists, namely, Bernhard and Rollier, insist that pigmentation of the skin may take place without previous inflammation, and Sonne concludes that although pigmentation may occur as the terminal event in photodermatitis, it may be produced without previous inflammation by grading the strength of the radiation. The basis of Rollier's method of treatment is the production of maximum pigmentation with complete avoidance of dermatitis. Hausser and Vahle (9) pointed out that the efficient wavelengths for both erythema and pigmentation were in the neighborhood of 300 $m\mu$, and claimed that the two things are very intimately connected, pigmentation following the action of particular wavelengths only when an erythema had been produced, although a slight erythema might occur without subsequent pigmentation. Coblenz, Stair and Hogue (2b, 2d) have brought together the outstanding work on the reaction of the untanned human skin to ultraviolet radiation. As Figure 3

shows, the wavelength range of the erythemogenic rays begins at about 315 $m\mu$, and extends to an undetermined wavelength shorter than 240 $m\mu$. The erythemic response curve rises steeply to a maximum at 297 $m\mu$, descends less steeply to a minimum at 280 $m\mu$, then rises to a less intense maximum in the region of 250 $m\mu$. The latest revision of the previously published data is shown in the following table, taken from Coblenz and Stair (2d):

TABLE I

The relative spectral erythemic response of the untanned human skin, of average pigmentation, to equal amounts of radiant energy.

Wavelength (in $m\mu$)	Erythemic Response	Wavelength (in $m\mu$)	Erythemic Response
240.0	0.56	289.4	0.25
244.6	.57	290.0	.31
248.2	.57	292.5	.70
250.0	.57	295.0	.98
253.7	.55	296.7	1.00
257.6	.49	300.0	.83
260.0	.42	302.2	.55
265.4	.25	305.0	.33
267.5	.20	310.0	.11
270.0	.14	313.0	.03
275.3	.07	315.0	.01
280.4	.06	320.0	.005
285.0	.09	325.0	.003
285.7	.10	330.0	.000

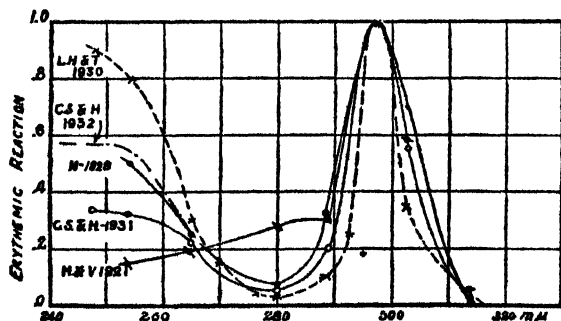


FIGURE 3. Relative spectral erythemic reaction of the human skin to equal amounts of radiant energy at various wavelengths; H. and V., Hausser and Vahle; L. H. and T., Luckiesh, Holladay and Taylor; C. S. and H., Coblenz, Stair and Hogue; H., Hausser. (Coblenz, Stair and Hogue).

The use of the erythema reaction has been emphasized by many as a means of appraising ultraviolet radiation. Keller (10) believes that the erythema reaction cannot be used to evaluate biological dosage because it is not sufficiently exact and probably cannot be made so, since it depends upon the time after irradiation that the erythema is observed, upon the part of the body irradiated, and upon the degree of erythema which has arisen. Coblenz, Stair and Hogue (2a, b, g), on the other hand, report an agreement, closer than 10 p.c., in the erythemic reaction of three sources differing very widely in their ultraviolet spectral distribution.

Ellinger (11) has studied the erythema reaction on more than 2000 subjects. His method consists in irradiating with a quartz mercury lamp the inner side of the forearm through 14 apertures in a template, closed one by one at 15-second intervals, the irradiation thus lasting for between 15 and 210 seconds. The number of ery-

thelial areas just perceptible 24 hours later is noted and the threshold in seconds determined.

There is great variability in the sensitivity of different individuals, the resultant of the influence of pigment, age, sex, and season.

1. Blondes are from 40 to 170 p.c. more sensitive than brunettes.

2. The sensitivity of children from 6 to 12 years of age is 50 p.c.; of young persons 13 to 19 years, 30 p.c.; of persons older than 50, 65 p.c. of that of those between 20 and 50 years of age.

3. Women are some 20 p.c. less sensitive than men.

4. In spring (March, April) there is a maximum, and in summer a minimum, following which there is a second maximum (October, November) (See Figure 4). The increase in the

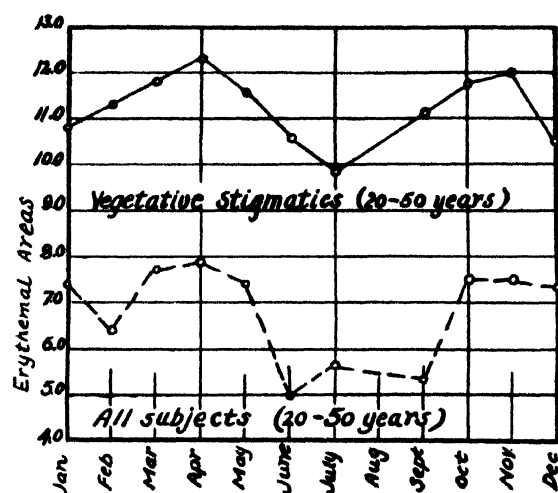


FIGURE 4. Seasonal variation in erythemic reaction (Ellinger).

sensitivity in March and April is probably due to increased thyroid activity, the diminution, beginning in May, to adaptation.

The sensitivity in some of the "light-sicknesses" is different from the average normal. In hydroa vacciniforme it is markedly diminished. In summer prurigo it is as markedly increased, and this may be pathognomonic. In xeroderma pigmentosum the sensitivity is very low. In psoriasis, in accordance with whether the patients do well or ill under light treatment, the sensitivity is low or high.

Early in his work, Ellinger met individuals with peculiar reactions, "vegetative stigmatics", or miniature Basedows, characterized by bright eyes, marked tendency to perspire, tremor, dermatographic tendency, psycholability, etc. In a second paper (11 b), he reports 93 observations on such persons, 12 men and 10 women, between

20 and 50 years of age. "Vegetatives" as a class are about 200 p.c. more sensitive than normals (see Figure 4), brunette "vegetatives" almost 360 p.c. and blondes only 50 p.c. more sensitive. The monthly sensitivity curve is always higher than the normal, even in summer, indicating a lesser degree of adaptation.

Von Bergmann has directed attention to the correlation between the incidence of gastric ulcers and vegetative stigmatism, and Ellinger stresses a further correlation based on increased sensitivity to the ultraviolet. Many of his vegetatives showed marked erythema upon exposure to sunlight, as well as to the quartz mercury lamp. We all know the striking action of histamine and of the H-like substances in the production of erythema. It is these H-substances which produce the reddening following irradiation designated as "light erythema", and in their transport from the erythematous area we have the basis for the frequently observed lowering of blood pressure following irradiation. The assumption that these substances, owing to their similarity to histamine, act also on the stomach, is a plausible one. As is well known, histamine brings about gastric hypersecretion and hypermotility, two important factors in the genesis of ulcers. There are numerous clinical and experimental observations illustrating this relation between histamine and ulcers.

The clinically observed increased incidence of ulcers in the spring and fall is interesting, occurring at times when the sensitivity, particularly of vegetatives, is so high. The spring peak is easy to understand because, apart from the increased skin sensitivity, there is a marked qualitative and quantitative increase in solar ultraviolet. That the incidence of ulcers does not increase in the summer when the ultraviolet intensity is high is due to the hot weather, which diminishes the secretion of gastric juice. This inhibitory climatic factor disappears in the fall, when there is again an increased skin sensitivity (see Figure 4), and a still quite considerable amount of solar ultraviolet.

The most logical reason for the increased light sensitivity of vegetative stigmatics is an endocrine imbalance, primarily involving the thyroid (Ellinger, 11 c).

Ellinger (11 d) has also studied the "photobiological constitution" in another group of abnormals. Occasionally he found an apparently healthy person with a very high sensitivity, due, as later examination showed, to a pulmonary tuberculosis. Observations were made on 128 persons of both sexes between 20 and 50 years old, with this disease. The material was subdivided into three groups on the basis of clinical and X-ray findings: (1) stationary (40 cases), erythe-

mal threshold of 210 seconds; (2) slightly active (43 cases), erythematous threshold of 120 seconds; and (3) very active (13 cases), erythematous threshold of 56 seconds. The normal average threshold is 113 seconds. The curve (Figure 5) shows

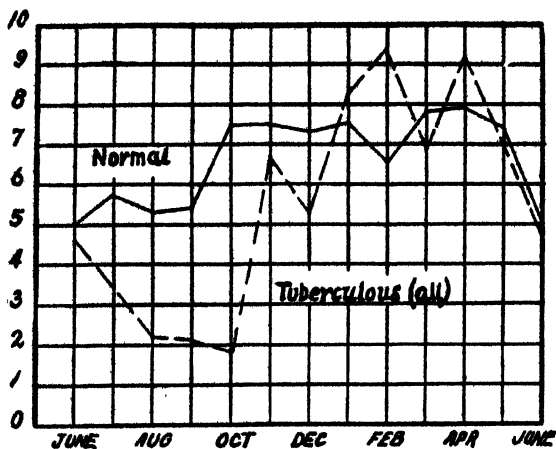


FIGURE 5. The seasonal sensitivity of normals and of tuberculous. (Ellinger).

a steep rise in the autumn which continues until the spring, in contrast to the healthy, to a peak much higher than the corresponding spring peak of healthy individuals.

The increased sensitivity of the active group is explicable on the basis of the action of H-substances. The active group is composed of patients in whom marked destructive processes are taking place, as a result of which protein decomposition products are set free, the vasodilating effect of which is well known. A peripheral capillary dilatation occurs in exudative forms of pulmonary tuberculosis. As a result of the high sensitivity of these patients, there is an increased action of radiation on the vascular system. Owing to the damage to the pulmonary vessels as a result of the tuberculous infection and their loss of elasticity, it is easy to see how a hemoptysis occurs, and why in the spring there is a marked increase in the number of fatal cases of miliary tuberculosis and of hemoptysis on the first brilliant sunny days.

Ellinger (11 e) has studied the erythematous reaction of women, normal, pregnant, "vegetative", and with menstrual disturbances. Three hundred and eighty-three "normal" women were studied, on whom 583 determinations were made. Placing the sensitivity of those between 20 and 50 years of age at 100 p.c., girls between 6 and 12 were 41 p.c., those from 13 to 19 were 20 p.c. and women over 50 years were 53 p.c. as sensitive. Blondes both light and dark, were some 36 p.c. more sensitive than brunettes, throughout the year. As has been pointed out before, women between 20

and 50 years are some 20 p.c. less sensitive than men of similar age. The sensitivity increases at the approach of the menses, a maximum being reached on the first day of the cycle, after which the sensitivity declines to the normal. During pregnancy the sensitivity is increased. Within the pregnant group there is no indication of the influence of age or of hair color.

The seasonal influence is apparent. The curves of the two groups (Figure 6) have the same

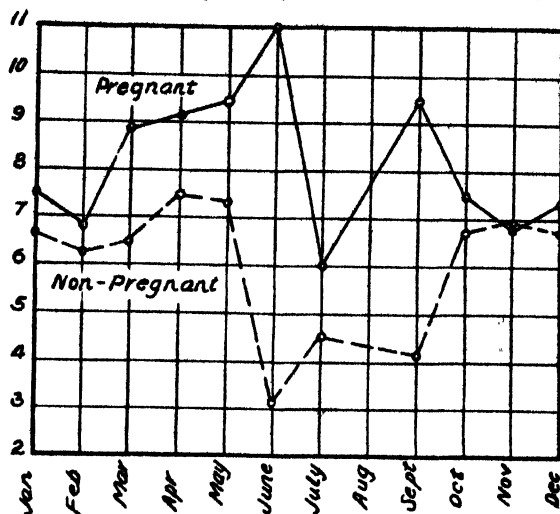


FIGURE 6. The seasonal sensitivity of pregnant and non-pregnant women. (Ellinger).

general form, a spring maximum followed by a summer minimum and a second fall maximum. But not only is the pregnant curve much higher, indicating a marked increase in sensitivity, but there is a shift in the position of the spring maximum to June, from April and May; of the summer minimum to July, from June; and of the fall maximum to September, from October and November.

Figure 7 shows the influence of the duration

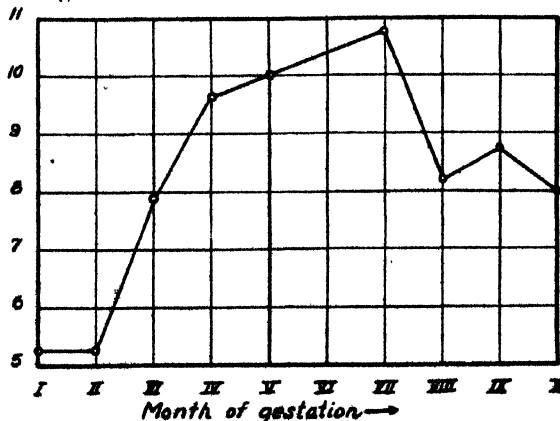


FIGURE 7. The influence of the duration of gestation on the sensitivity of women. (Ellinger).

of pregnancy on the sensitivity, increasing from the third month to a maximum in the seventh, and then falling again.

Ellinger (11 e) studied 17 women (20 to 50 years old; 3 blondes and 14 brunettes) with the stigma of vegetative imbalance. Independent of hair color there is an increase in sensitivity of some 200 p.c., particularly evident in the brunettes who on the average throughout the year show an increase of 265 p.c. (Figure 8). A few

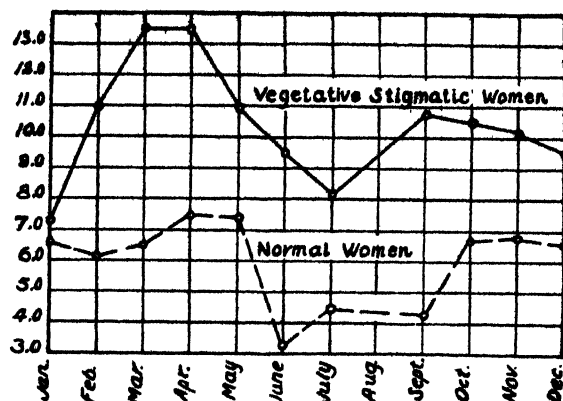


FIGURE 8. The seasonal sensitivity of normal and of vegetative stigmatic women. (Ellinger).

observations on persons under 20 or over 50 also showed some increase in sensitivity. In contrast to earlier studies where the influence of sex was not considered, and in which for all vegetatives a value of +250 p.c. was given and for the brunettes alone a value of +360, we now see that the sex difference (less sensitivity for women than for men) holds, for in the vegetative women the values are respectively 200 and 265 p.c.

Ellinger (11 f) seeks to explain the marked variations in sensitivity on the basis of the number of open skin capillaries. Table II is based on 88 cases distributed in age between 19 and 50.

TABLE II

Group	Light Sensitivity	Threshold in sec.	Capillaries per sq. mm.	No. of Subjects
1 Blondes	7.3	101	35.0	28
2 Brunettes	5.0	135	35.6	15
3 Men	7.3	101	36.0	24
4 Women	5.5	128	34.2	19
5 Pregnant Women	9.4	69	48.8	39
6 Men and Women	6.5	118	35.2	43
7 Vegetative Stigmatics	10.4	54	38.0	6

The table shows the following: (1) the difference in light sensitivity between blondes and brunettes (Groups 1 and 2) cannot be correlated with a difference in the number of capillaries, being due to the difference in pigment. (2) On the other hand, the difference in the sensitivity between men and women (Groups 3 and 4) parallels the difference in the number of open capillaries. The differences are clear enough so that they have significance, although they cannot be statistically treated. (3) There is no doubt about the increase in sensitivity in pregnant women being paralleled by an increase in the number of capillaries (Groups 4 and 5). (4) The increase in the sensitivity of the vegetative stigmatics is possibly paralleled by an increase in the number of capillaries (Groups 6 and 7). In a case of porphyrinuria with a high threshold (270 seconds), the number of open skin capillaries was only 20 per sq. mm.

THE FUNCTIONS OF PIGMENT

The essential function of the skin pigment, melanin, has for long been thought to be the protection it affords against excessive irradiation. Finsen is regarded as having demonstrated this protective action by showing that on successive exposures of the arm to the energy emitted by a carbon arc, the pigmented areas were not inflamed, while those areas previously covered by glass and thus not pigmented, showed an intense reaction. Everyone has observed how the skin of persons accustomed to an outdoor life, and thus tanned or pigmented to a greater or less degree, does not react on exposure to sunlight which acts painfully on the unaccustomed, unpigmented skin of others. Instances have also been cited of physiological responses to irradiation ceasing after pigmentation, such as changes in the blood picture, in blood pressure, blood sugar and serum calcium. Pigment also affords protection against visible radiation in cases of photodynamic sensitization in animals.

The ectodermal pigment is found in the epidermis, almost exclusively in the basal cells, chiefly in characteristic cap form over the distal pole of the nucleus, but also in branched, dendritic-like cells (Bloch 12). The cells of the malpighian and horny layers in the white race contain pigment in considerable amount only when pigmentation is extreme (Figure 9). In negroes not only is pigment more abundant in the basal layer, but there is also much pigment in the outer, including even the horny, layers (Figure 10). The mesodermal pigment is in the corium, or cutis vera, and there are two entirely different kinds of cells. One is found regularly, but in varying numbers, in the papillae and in the deeper

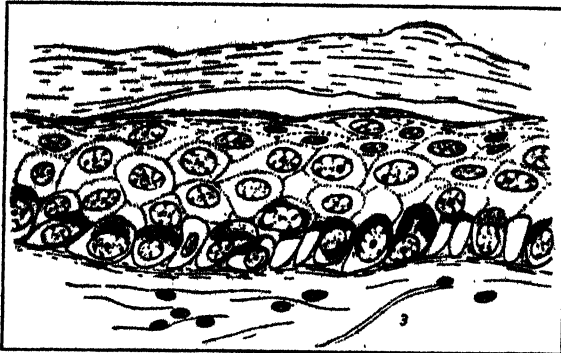


FIGURE 9. Drawing of section of skin of blonde ("cross between blonde and brunette"). In lighter blondes, fewer basal cells contain still fewer granules; in darker brunettes the basal cells contain more granules. (Jordan, H. E., *Am. Naturalist*, 45, 452, 1911.).

layers. They are plump, sometimes somewhat branched cells of very variable form containing coarse granules of pigment. These are the chromatophores of the human cutis (Figure 11). They do not form their pigment themselves, but are connective tissue cells which have phagocytized pigment, originally formed in the epidermis. The other type of pigmented cell in the cutis differs entirely in form, localization and nature from the chromatophores. These are mesodermal melanoblasts (Mongolen Zellen) and are generally long and ribbon-like. They elaborate their own pigment entirely independently of the epidermal pigmentation and, embryologically, long before this has appeared.

The cutis normally contains only traces of pigment which have arisen in the epidermis and which have been taken up secondarily by the chromatophores. Marked heaping of cutaneous



FIGURE 10. Drawing of unstained section of negro skin, showing the distribution of the pigment granules in the epidermis. (Jordan, H. E., *Am. Naturalist*, 45, 450, 1911.).

pigment is found only in pathological processes, resulting in damage or destruction of the pigment-containing epidermal cells (reaction to radiant energy, to X-rays, precancerous conditions, etc.)

Considerable evidence has been brought against the view expressed by Finsen, and quite universally held since his day, that a pigmented skin was protected against excessive irradiation with radiant energy shorter than about 315 m μ . The primary damage, as indicated by the inflammatory reaction, is to the living epidermal cells, the prickle cell layer (rete mucosum of Malpighi), and not to the vessels of the dermal papillae. But the pig-

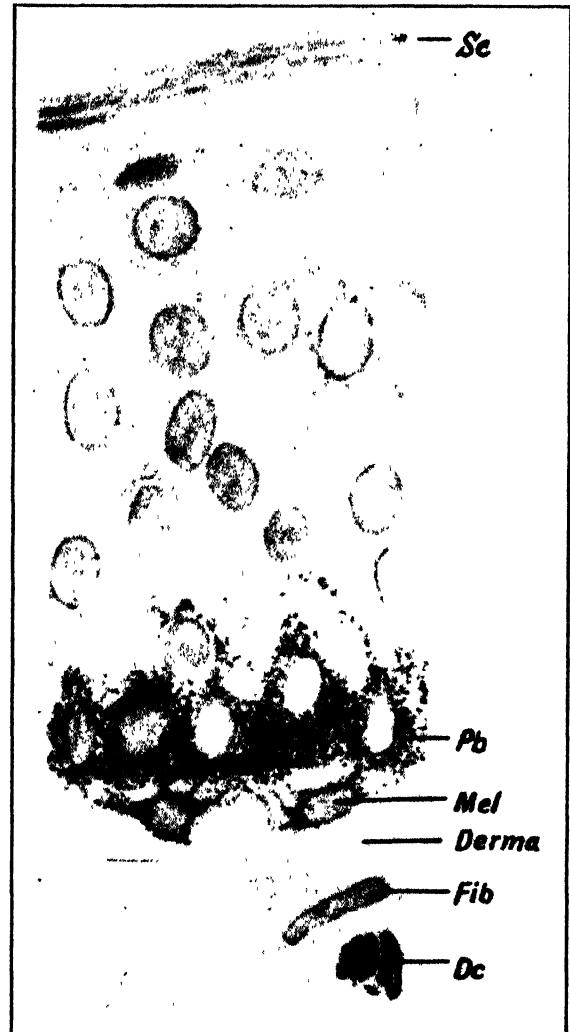


FIGURE 11. Section through the skin of a human mammary papilla: Sc, Stratum corneum; Pb, pigmented basal cells of epidermis; Mel, melanoblasts; Fib, fibroblast; Dc, dermal chromatophore. (Maximow, A. A. and Bloom, W., *A Textbook of Histology*, 1934).

ment is almost exclusively in the epidermal basal layer, below the prickle cells, and does not push into and over this layer until some days after the inflammatory reaction (see Figure 9). Furthermore, a vitiliginous skin, without pigment, may become accustomed to irradiation and no longer show an erythema when irradiated with short wavelengths.

Meyer and Bering concluded that the horny layer filtered out the short waved ultraviolet wavelengths which are harmful in too great amounts, so that this layer represents an important protector of the living epidermal cells. Miescher (13) showed that the reparatory process in such inflamed skin consists in proliferation as well as in cornification, so that the horny layer is thickened, and thus the amount of short waved, erythema-producing radiant energy which reaches the living epidermal cells is diminished. According to this, adaptation to short waved irradiation is the direct action of a reactive proliferation and thickening of the horny layer. The protective action depends on dispersion, reflection, but mostly on absorption.

Owing to this demonstration of the protection afforded by the thickened horny layer, some persons have come to deny to skin pigment any function as a protection against ultraviolet wavelengths, and regard it as merely a heat filter and localizer, in the absorption of luminous and infrared rays. But melanin is a strong absorber of ultraviolet radiant energy. A 0.3 p.c. solution of cuttle fish melanin absorbs about twice as much as an egg albumin solution of similar concentration. A solution of dopamelanin is about as efficient an absorber as is quinine in equal concentration, and some five to ten times better than keratin from white sheep wool and white human hair (Meyer and Kirchhoff, (14)).

The following is what may be called the modern point of view concerning the protective function of the skin against radiant energy (13, 14). The organism is protected from over-irradiation by ultraviolet by two mechanisms. The shorter waved rays are absorbed superficially in the horny layer (about 30 $m\mu$ thick), and thus never reach the living cells of the rete mucosum and basal cell layer: *the horny layer is the screen for the epidermis against the shorter ultraviolet rays.* The longer waved ultraviolet which penetrates as far as the cutis (at least from 50 to 80 $m\mu$) may thus act on the blood in the capillary vessels of the dermal papillae. The basal layer pigmentation, which occurs after irradiation, regulates the amount of this longer waved energy which reaches the basal cells and thus protects the underlying cutis from receiving too much energy: *pigment is the screen for the cutis against the longer ultraviolet rays, screening the papillae.* As

a matter of fact, the function of skin melanin as a screen against the ultraviolet is relatively small in the white race, of more importance in dark-skinned races and is the principal reason for the low sensitivity of the negro to ultraviolet.

Quite a number of authors believe that this pigment represents a filter for the penetrating luminous and near infrared rays so that the heat effect is localized at the surface, from which it may be more readily lost by radiation and evaporation, thus protecting the body as a whole from overheating. The advantage enjoyed by dark-skinned persons over fairer ones in a hot climate in the way of greater tolerance to the rays of the sun is thus plausibly assumed to be demonstrated. The negro's skin heats sooner and to a greater extent than does that of the white man, he therefore sweats more copiously, and the sweat evaporates more quickly, owing to the higher temperature of the skin.

All of this, however, while plausible, is almost all assumption. As Miescher (13b, c) points out, no careful and exhaustive studies have been made of the influence of pigment on heat regulation in fair and dark skinned persons and, from his own observations, he believes that the pigmented skin of the negro is not very advantageous to him in this way. He concludes that the pigment in the skin of man absorbs to varying degrees all of the wavelengths of radiant energy. Its protective function is but slight in white skinned persons, more important in dark skinned races, particularly for the ultraviolet rays. The increased absorption by pigment of penetrating heat rays does not appear to give the negro any advantage.

THE THERAPEUTIC VALUE OF PIGMENT

There is no unanimity of opinion concerning the therapeutic value of pigment. Many regard pigment, owing to its absorption, as unfavorable for the action of radiant energy and therefore attempt to prevent its formation. Others ascribe to it a very important value and believe that the degree of pigmentation is a favorable diagnostic sign, brunettes thus responding better to insolation than blondes. Pigment formation and healing seem to represent independent, coordinate phenomena proceeding simultaneously and in the same direction. Since the horny layer is a more important protector than is skin pigment against over-irradiation by shorter ultraviolet rays, there is left but one outstanding significant connection between radiation and pigment, namely, as an indicator of the action of the radiant energy, its intensity being, to a certain extent, proportional to the amount of action. But it is also very dependent upon individual factors such as race, constitution and body function. Pigment formation is an indicator of the desired action and can be used as

a measuring rod for therapeutic treatment, and, since pigment formation, horny layer thickening, and possible chemical alterations of the skin cell proteins (15, 16) run practically parallel, it is also a measure of adaptation, or of diminished sensitivity.

THE EFFECT OF RADIANT ENERGY ON WOUNDS AND ON SOME SKIN DISEASES

Natural sunlight will hasten the healing of sluggish, indolent wounds, as was demonstrated so clearly by Oscar Bernhard (17) at St. Moritz. The effect, however, is certainly not specific to the short ultraviolet rays, but is brought about by wavelengths that penetrate through the epidermis and part of the dermis, producing their action indirectly through the circulation. The surface action of ultraviolet wavelengths shorter than 290 $m\mu$ or of those slightly longer (up to 310 $m\mu$) in large quantity is detrimental to the healing of wounds, unfavorably influencing the processes of repair. The beneficial influence is due to longer ultraviolet, luminous and infrared rays, and so the quartz mercury vapor lamp is not indicated, while natural sunshine and the energy from "Sunshine" carbons are. Coulter and Smith (18) believe that ultraviolet radiation has a definite value in the treatment of wounds and Pollaczek (19) recently reported on the beneficial use of artificial energy.

Gauze soaked in irradiated ergosterol in liquid paraffin (20) is efficacious as a dressing for ulcers, refractory and granulating wounds, the healing of which has been delayed by infection.

Until Finsen, by means of strong carbon arc radiation, cured so many cases of tuberculosis of the skin (about 60 p.c. of those he treated) the disease had been regarded as almost incurable. Reyn (21) has found that local treatment combined with general exposure is beneficial in approximately 90 p.c. of cases. The result of the treatment depends, as in the treatment of wounds, upon the depth of the action of the effective rays and, in selecting the source, the one with the maximum percentage of these rays must be chosen, namely, the carbon arc lamp.

Lupus, as we all know, is also treated by means other than radiant energy or than radiant energy alone. Mention may be made of ointments, of diet, of borderline X-rays, of electrocoagulation and of surgery. Lomholt (22) has recently summarized the treatment as carried out at the Finsen Institute.

Coulter and Smith (18) and MacKee (23) have reviewed the evidence for the use of ultraviolet rays in dermatology. Of skin diseases or dermatoses for which definite claims are made for the beneficial action of sunlight and artificial radiation the following may be cited: dry and weep-

ing eczema, pruritus, local and generalized urticaria, psoriasis, acne vulgaris, varicose ulcers with associated dermatitis, vascular nevi, alopecia areata and the loss of hair following severe infections. Lupus vulgaris, however, seems to be the only skin disease on which ultraviolet rays act specifically (24). There are numerous other diseases of the skin in which local or general exposure may have a favorable action, but the improvement that may follow is not a specific effect of these rays. In some cutaneous disorders (eczema, psoriasis, lupus erythematosus, herpes simplex, xeroderma pigmentosum, farmer's or sailor's skin, prematurely senile skin) exposure to such rays may cause an exacerbation, provoke an attack, or produce other injurious effects.

THE BLOOD AND CIRCULATION

The influence of radiant energy on the blood may be passed over briefly, since, while radiant energy, including longer ultraviolet, luminous and infrared rays such as natural sunlight and its close approximation—the energy emitted by "Sunshine" carbons—may have some effect on secondary anemia, this is limited, not specific, and merely adjuvant to established dietetic and drug treatment (1, p. 188).

Ultraviolet rays alone do not lower the blood pressure, but carbon arc radiation ("Sunshine" carbons) does—that is to say, of between 60 and 70 p.c. of persons with abnormally high blood pressure (essential hypertension). Laurens (1, p. 168) has shown in both ambulatory and hospitalized patients with essential hypertension that both systolic and diastolic pressure may be materially reduced (from 10 to 15 p.c.) by general carbon arc irradiation. The "cure" is temporary, and the treatments have to be repeated, being given every ten days or two weeks in those whose pressures have been lowered by earlier more often repeated large doses (usually every 4th or 5th day). The individual doses must be large enough to set up an inflammatory reaction, accompanied by marked vasodilatation, not too frequently repeated, so as to avoid pigmentation. Such treatment will produce a 10 to 15 p.c. reduction in both systolic and diastolic pressures. The mercury vapor lamp in quartz will not do this and the effect is thus not the specific effect of ultraviolet rays but of these and, in addition, the luminous and infrared, as emitted by "Sunshine" carbons.

MINERAL METABOLISM

Rickets. Ultraviolet rays shorter than 313 $m\mu$ are of great importance in rectifying the partial lack of the dietetic components necessary for building bone. The process of irradiating a baby

with such ultraviolet wavelengths consists in giving rise to vitamin D from the provitamin in the skin. The irradiation influences the storage of calcium and phosphorus and the equilibrium of these elements in the blood stream of mature animals in a way similar to the effects upon growing animals and the antirachitic factor, or vitamin D, represents specifically the organic agent which promotes normal calcium metabolism. It may prevent and cure rickets, it may promote growth, or it may simply prevent excessive loss of lime from the body. The specific capacity in which it functions depends upon the condition of the organism, both with respect to age and nutrition, and upon the composition of the diet. Radiant energy simply revives, or aids, a depressed function. The antirachitic vitamin D is prepotent in preventing rickets, and is capable of doing so in the entire absence of radiant energy. Ultraviolet radiation may rectify partial, but not absolute, lack of the dietetic components necessary for bone and teeth calcification. Ultraviolet radiant energy and vitamin D cause the organism to operate more economically, they make metabolism more efficient, they permit the organism to have full use of normal processes which are not effective, but they do not bring new processes into operation (1, p. 285, 389) (25).

Fractures. Ultraviolet, plus luminous and infrared radiation, does not favorably influence the union of fractures.

Teeth. Diet influences the formation of teeth by virtue of vitamin D, or of ultraviolet radiation, which increases and controls the actual calcifying process, and by containing sufficient calcium and phosphorus. The vitamin seems necessary not only for the original development of the tooth but for its protection later in life (26, 27). In dental caries, rickets seems to be merely one of several etiologic factors. Dental caries is not the result of low calcium or low phosphorus content of the blood or saliva (28, 29, 30). A comparative study of enamel, dentin and bone in new-born and very young infants shows quite clearly that the formation of enamel and that of dentin in the unerupted teeth do not parallel each other, but that those of bone and dentin do (31). Enamel is an epithelial tissue arising in the ectodermal layer of the embryo, while bone and dentin are connective tissues originating in the mesoderm. This may be why the teeth of children with marked stigmas of rickets are often well formed and free from decay, and why tooth decay may be rampant in rapidly growing, breast-fed infants in the tropics, with no evidence of rickets.

Milk in Rickets. As human milk or that of some animal forms the almost universal diet of infancy, it has been surprising to learn that ric-

kets can occur so frequently. One expects milk above all foods to embody the essentials for good health and development during infancy. But milk is poor in the antirachitic factor, vitamin D, of which even the highly prized human milk contains only a small amount. Since the introduction of irradiated ergosterol and irradiated foods for the prevention and treatment of rickets, several methods have been satisfactorily employed in order to impart antirachitic properties to milk. Among these may be mentioned: (1) The irradiation of milk in both the powdered and liquid forms; (2) the irradiation of the mother or of the wetnurse; (3) feeding to the cow irradiated yeast, and to the woman cod liver oil or irradiated yeast; (4) adding irradiated ergosterol (yeast) to the milk; (5) adding to the milk a vitamin D concentrate prepared from cod liver oil.

Infantile Tetany. Infantile tetany is a symptom complex which occurs in rickets when the salt equilibrium in the blood, namely, low calcium, happens to be of a kind which sets the nervous system in a state of hyperexcitability, and it is with the low calcium form of rickets that manifest tetany is associated. Any agent (such as cod liver oil, the antirachitic wavelengths, etc.) capable of raising the calcium concentration to a level within 20 p.c. of the normal will cure the active manifestations of tetany. The treatment of choice is a combination of calcium chloride and irradiated ergosterol. Completely parathyroidectomized rats, dogs and men may be kept symptom-free by large doses of irradiated ergosterol (33, 34, 35).

ACTIVATION

Activation is one phase of "photochemistry in medicine" that all of us know something about merely by virtue of the fact that there has been so much written about it (1) p. 389, (36). The independent and almost simultaneous demonstration in 1924 by Hess (37) and by Steenbock and Black (38) that certain substances, inert in so far as calcifying and growth promoting power are concerned, may have these capabilities bestowed upon them by irradiation has proven to be of far reaching importance. The facts that when an animal is irradiated its skin, liver and muscle become antirachitically active, and that eggs, milk and feces have increased or newly endowed calcifying and growth promoting powers bestowed upon them, and that diets deficient in the antirachitic vitamin may have it supplied to them by judicious irradiation, have proved not only of interest and appeal to the layman and of the greatest scientific and therapeutic value, but have done much toward advancing our conception as to the mode of action of ultraviolet radiation

and of vitamin D in the prevention and cure of rickets.

The identity of the influence on metabolism of exposure of the skin to radiation and the administration by mouth of cod liver oil or of a substance that has had its vitamin D content influenced by being irradiated is interesting. Radiation forms this substance either in the cells of the living creature or in its foodstuffs. The action of a foodstuff artificially rendered antirachitic by irradiation is identical with the action of a naturally occurring foodstuff containing the antirachitic factor.

The use of irradiated milk (fluid, dried, evaporated or condensed) is proving to be one of the important prophylactic developments of "activation" (39, 40). Of less importance is the activation of foodstuffs (cereals, bread, etc.). A fact of broader interest than the prophylactic and curative value of irradiated milk is the clear demonstration of the superior clinical effectiveness of irradiated milk to cod liver oil and to viosterol, as well as to the milk from cows fed irradiated yeast. It would seem (41, 42) that the effect of irradiating milk with ultraviolet rays is to produce a product of peculiar efficacy in the treatment of human rickets. Perhaps, the combination of provitamin D with the milk proteins may enhance the usual effect of irradiation on this sterol. There has been evidence for some time that vitamin D produced by irradiation of ergosterol is a different substance from that found in cod liver oil (36).

The Chemistry of Vitamin D. This, an outgrowth of the demonstration of the potentiality of being activated, has been most intensively investigated. Most persons think that ergosterol is the parent substance or provitamin of vitamin D, being converted into the antirachitic vitamin, when properly irradiated. A few (notably Bills and Koch) believe that vitamin D activity is not limited to ergosterol, but that it may be a general property in varying degrees of various sterols ((1) pp. 407-409, 435) and ((36) pp. 36, 43) and that there are at least three forms of vitamin D. Waddell (43) has recently championed the view that the activatable provitamin constituent of cholesterol is a substance different from ergosterol (see also Hathaway and Koch, (44)).

The toxicity of irradiated products may be passed over very briefly because nowadays, in order to produce toxic effects, or signs of hypervitaminosis D, dosages thousands of times larger than therapeutic must be administered. (This subject is discussed at length in my monograph (1) p. 435 and by Bills (36) p. 52).

The experimental work on hypervitaminosis D has, however, been of service in suggesting views as to the mode of action of vitamin D, and the part played by the richness or poverty of the diet in calcium and phosphate on the working of the factor that "mobilizes" these substances. When the diet is deficient in calcium the withdrawal from the bone stores becomes the noteworthy feature of the hypervitaminosis. When the diet is rich in calcium the bone is less called on, but there is increased liability to calcareous deposition as a result of an increased net absorption from the intestine, and partly also, no doubt, because the amount stored by the bone is less than normal. Each addition of calcium to the diet intensifies the hypercalcemia and calcareous deposition with a given overdose of vitamin D.

Passing mention should be made of the fact that vitamin D sclerosis is a lesion of the media of the arteries, intimal changes being of secondary nature and non-essential. The essential vitamin D lesion consists of muscular degeneration with calcification of the media. For a review of the histopathology of hypervitaminosis D, see (1) pp. 450-471.

The question has been repeatedly discussed as to whether it is the antirachitic substance in irradiated ergosterol which has this toxic effect or some other irradiated product present as an impurity. To an unbiased observer the evidence indicates that the two effects are produced by the same substance, in other words, that the toxicity is an inherent property of the "pure" vitamin (45, 46, 47, 48, 49).

A number of investigators see similarity in the rise of blood calcium produced by excessive doses of vitamin D and the action of the parathyroid glands (see, e.g., Taylor, Weld and Sykes (50)). As Dale, Marble and Marks (51) have indicated, the resemblance between the effects of vitamin D in excess on a diet poor in calcium, and those produced by excess of the parathyroid hormone are fortuitous. Similarly the effects of excess vitamin D and of parathyroidectomy on blood calcium are in opposite directions but not connected by any causal relationship.

PHOTODYNAMIC OR OPTICAL SENSITIZATION

It is possible to sensitize living cells, just as one sensitizes a photographic plate, and produce an abnormal condition in which light is as active as ultraviolet. This is photodynamic or optical sensitization. It was discovered accidentally by Raab (52) who, under the direction of von Tappeiner, was studying the toxic effect of acridine on *Paramecia*. Discordant results in determining the minimum fatal dose led to the discovery that acridine is lethal only in the light. With a

strength of 1:20,000, the *Paramecia* were killed in 6 minutes in direct sunlight, in 1 hour in diffuse daylight, and were unharmed in the dark. After this discovery the subject was extensively investigated by von Tappeiner, Jodlbauer and their co-workers (53) and many substances were found to act as sensitizers, fluorescein and its derivatives being especially potent. *In vitro*, a surprising number of interesting results was obtained and it was found possible to sensitize to the action of light, bacteria, protozoa, red blood corpuscles, enzymes, ferments, the various substances concerned in immunity and certain well-defined chemical substances. Fluorescence is the usual accompaniment, but not the fundamental cause of, the sensitization.

Photodynamic sensitizers arising under physiological as well as pathological conditions are capable, in the presence of light, of destroying both warm and cold-blooded animals. There are illnesses which affect man and animals when they are exposed to light, either because the light is very strong, or because of a hypersensitivity, due to exogenous or endogenous causes.

The usual meaning attached to photodynamic sensitization is the production by light plus a sensitizer of the same reaction that ordinarily takes place under ultraviolet. A very considerable difference, however, exists; whereas the ultraviolet effects can occur either in the presence or absence of molecular oxygen, the photodynamic effects occur only in the presence of oxygen. Sensitization, however, may also be induced in the ultraviolet as Hausmann and Rosenfeld (54) and Kuen and Rosenfeld (55) have demonstrated for red blood cells and Lassen (56) for *Paramecia*.

For detailed discussion of sensitization in animals and in man the chapter in my monograph on "The Physiological Effects of Radiant Energy" (1) p. 488, ff. and the review by Blum (57 a) should be consulted. Among recent papers may be mentioned those of Blum and his co-workers (57 b-e) and of Perdrau and Todd (58).

Photodynamics has not proved a fruitful field therapeutically although attention has been directed to acceleration or increased efficiency in radiation in conjunction with a sensitizer (59, 60, 61). Szczygiel and Clark (62) have recently demonstrated, however, that there was no evidence of prevention of rickets in rats exposed to visible energy after ingestion of eosin. Attention has also been directed toward the desensitization of patients to light in acne, variola, hydroa vacciniforme, and in eczema solare (63, 64).

Pathologic Conditions Produced by Sensitization. In addition to buckwheat sickness in cattle, the condition seen in sheep after eating *Hy-*

pericum crispum, (St. John's wort), probably also the clover sickness, and the illness of swine following the eating of *Lachnanthes*, etc., are examples of exogenous sensitization. Chlorophyll has never been shown to produce such a condition.

One of the most interesting and perfect examples of exogenous photodynamic sensitization in man is that described by Prime (1), p. 505) of twenty-six cases in which eosin (containing 47 p.c. bromides) was given by mouth for epilepsy. Jausion (63) has described several cases of acute sensitization following treatment with tryptaflavin (acriflavin), and Hausmann (65) cites cases of skin sensitization in patients taking luminal.

Amongst conditions found in man presumably or possibly due to endogenous sensitization are the following:

(1) *Hydroa estivale* or vacciniiforme (Blum (66) Hausmann (67), Gottron and Ellinger (68)). It is quite possible that the porphyrins play no part in the light sensitivity, or may be products of skin injury.

(2) *Eczema solare* (1) p. 510) is an example of a condition due to increased sensitivity to light, for which the cause is unknown.

(3) *Prurigo estivale*, or summer prurigo, a severe, relapsing bullous eruption, occurring in warm weather only, has been thought by some to be due to a harmful influence of direct solar irradiation on sensitized skin. Jesionek, among others, doubts that light has anything to do with it.

(4) *Pellagra*. Payne and Perlzweig (69) discuss the relation between abnormal sulphur metabolism and pellagra. Anderson and Ayres (70) think that light sensitivity is bound inseparably with sulphur metabolism. See also Smith (71). There is probably no connection between the etiology of pellagra and sensitization in man.

(5) *Xeroderma pigmentosum*, a rare and fatal disease, characterized by brown spots and skin ulcers, with muscular and cutaneous atrophy and telangiectasis. The first acute signs occur on the early brilliant days in spring, and consist of red spots, for the most part on the face, and seem to be the result of direct exposure to sunlight. According to Lynch (72) the course of *xeroderma pigmentosum* is progressive, the exposed parts of the skin undergoing all of the progressive changes of senility; as a rule, death results from carcinoma (73, 74, 75, 76). Ephelides, seaman's, sailor's or farmer's skin and senile skin are all characterized by the same lesions and produced by the same external agents, and some observers have regarded them as forms of *xeroderma pigmentosum*. The late changes resemble roentgen-ray dermatitis clinically. Bernhard pointed out

that, although sailor's skin occurs frequently in those who are out in the open air at high altitude (Swiss Alp guides, etc.), he has never seen a case of skin carcinoma in such persons.

(6) *Lupus erythematoses discoides* (L. erythematosus) is regarded by some as one of the dermatoses caused by light. It usually affects only the uncovered parts of the body (70, 72, 74).

Greenbaum (77) described the cutaneous dangers of ultraviolet irradiation in children. Prolonged exposure to the violet and ultraviolet rays of the sun, and to those artificially produced, may cause not only systemic disturbances but also inflammatory and degenerative changes in the skin, varying with the person. The harmful systemic effects have not been well understood but deaths of infants following short exposures to ultraviolet radiation have been reported. Hausmann (78) also sounds a warning against the dangers of over-indulgence in radiation. He believes that, as the situation stands, there is little danger that people on the whole are getting too little sunshine, but rather that they get too much, and he has no doubt but that this is going to give rise to a number of acute and chronic pathologic conditions, if these are not present already. He has in mind particularly the central nervous system, especially the brain, which he thinks must be injured by the over-irradiation and resultant heating of the skull. Hausmann also points out that although the skin may become accustomed to sunlight, it is certain that its action on certain parts of the skin results in a predisposition to certain conditions. An outstanding example is the well known reddening on the chest of women, designated by Brocq as "Dermatose du triangle sternoclaviculaire". Brocq demonstrated that this conditions a *locus minoris resistentiae* for several skin diseases, such as acne, urticaria, eczema, which if not due directly to the action of light, are indirectly so. Klare (79) has described a febrile bronchitis manifesting itself in children with exudative lymphatic diathesis. They are usually of light complexion with hair of a slight reddish tone. The skin reacts with inflammation and burning, not with pigment formation.

TUBERCULOSIS

Heliotherapy lays claim to at least two triumphs, the cure and prevention of rickets, and the treatment of extrapulmonary tuberculosis, but there are numerous points, particularly in the latter, which are far from settled. As Luce-Clausen (80) points out, on reading Rollier's book on heliotherapy (81), one is impressed with the results obtained, especially in bone tuberculosis, by a treatment régime in which helio-

therapy in the form of the complete sun bath is one of the factors employed. But, while heliotherapy undoubtedly plays a large part in the results reported at Leysin, it is only a part, not the whole, of the régime. It is at present impossible to evaluate the other factors such as exposure to exceptionally dry and pure air, freedom from fog, dust, winds and rain, combined with ideal conditions of feeding, prolonged periods of rest, orthopedic treatment, and wherever possible, pleasant occupational therapy. These, in themselves, must play a large part in the excellent clinical results obtained.

Tuberculosis, wherever situated, is a general disease presenting local manifestations, and as such demands treatment directed towards the improvement of the general health. The object of the sun treatment is to place the weakened and diseased bodies of patients in an environment giving the greatest possible assistance in the fight against tuberculosis. In this environment the essential factors are the air of high altitude and the sun bath applied to the whole surface of the body. Rollier regards heliotherapy as a local treatment which is at the same time analgesic, bactericidal, and a powerful stimulus to cicatrization. In choosing the High Alps for its application, he was guided by the fact that the climate of these regions is practically the only one in which heliotherapy may be applied with advantage in every month of the year. At an altitude of 1500 meters the air is never oppressively hot, even in the height of summer; in winter, although the atmosphere is intensely cold, the intensity of the sun's rays more than counteracts this quality. Rollier is convinced that, correctly understood and applied, heliotherapy fulfills the highest demands of orthopedics and conservative surgery. (see Bernhard (82 a, b, c), Jesionek (83)).

Numerous contra-indications have been advanced to heliotherapy in phthisis, but the only valid one is met with in all localizations of the disease. When a patient is very weak, with much toxemia and hectic fever, the sun bath is liable to do more harm than good, and should therefore be deferred with the hope that rest and fresh air will strengthen the patient sufficiently for insolation to be justifiable at a later date. The combination of hot air and intense sunlight is bad for any form of tuberculosis, though particularly dangerous in the pulmonary variety. Both solar and air exposures can be employed for mildly active forms of pulmonary tuberculosis, chronic and having little or no fever; but it is preferable that the exposures should be made to diffuse daylight or to sunlight of low intensity. The very active or progressive forms of pulmonary tuberculosis, particularly those accom-

panied by high fever, are contra-indications to solar exposure. Even in the rather stationary forms very intense sunlight, especially in overdoses, can incite harmful focal reactions (See Rollier (81); Bernhard (82); Mayer (84)).

Mayer (84 b) emphasizes a fundamental point of view: "For the extrapulmonary forms of tuberculosis physiotherapeutic measures often prove valuable adjuvants, but they are never to be employed to the exclusion of rest and hygienic-dietetic, and perhaps surgical, measures. Light in any form by itself is not curative but may prove an important aid. To believe that light by itself will cure tuberculosis, to be unduly optimistic about its effects and to consider it a specific form of treatment, or to use it without sound medical advice and employ it to the exclusion of rest, hygiene and diet, eliminating orthopedic measures or the occasional necessary surgical intervention in bone and joint tuberculosis, is bound to bring criticism to an otherwise eminently desirable method of treatment."

Many specialists feel that heliotherapy constitutes a valuable aid to the fresh air cure in the treatment of pulmonary tuberculosis and that it can be practiced everywhere, but particularly favorably at the seashore or in the mountains. It is best carried out, however, in the climate most suitable to each individual case (see Roussel (85); Gauvain (86); Eliason (87); Freiberg (88); Phelps (89); Conrad et al (90); Coulter and Smith (18)).

There are numerous reports on the various manifestations of tuberculosis, pulmonary, pleural, laryngeal, lymphatic, intestinal, peritoneal, genito-urinary, bone and joint ((1) p. 522 and (84 c.)). Some of the results are very favorable, indicating that the "light treatment" or irradiation, is an important aid to hygienic treatment. But reliance on radiant energy as an important aid in pulmonary tuberculosis is to be discouraged, despite the claims of many phthisiologists. In isolated instances when the pulmonary tuberculosis is of the fibrous form approaching arrest and showing only slight or no elevation of temperature, improvement is occasionally seen. Of outstanding importance is the realization of the fact that heliotherapy, when abused, is dangerous to the tubercle.

CONCLUSIONS

The number of human ills and abnormalities for which radiant energy, as emitted by the sun, carbon arcs and quartz mercury vapor lamps, is specific or adjuvant is small, much smaller than the number for which claims are made by manufacturers, as well as by members of the medical profession.

Ultraviolet rays (shorter than 313 $m\mu$) are specific in the cure and prevention of rickets (infantile and adult) and infantile tetany. Such wavelengths are not always available in sunshine, and recourse to artificial sources is frequently necessary.

Cases of extrapulmonary tuberculosis, including lupus vulgaris, are markedly benefited by careful exposure to sunlight or to the closest approximation to natural sunlight; viz., the energy emitted by a flaming carbon arc lamp. The benefits are due not solely to ultraviolet rays, either those specific in rickets or somewhat longer, but as well to the light or luminous rays and to the heat or infrared rays, which are so preponderantly present in natural sunshine and carbon arc radiation. The reports of the results with the quartz mercury lamp in the treatment of extrapulmonary tuberculosis are disappointing.

Natural sunlight will hasten the healing of sluggish, indolent wounds, as demonstrated by Oscar Bernhard at St. Moritz. This effect, however, is certainly not specific to the short ultraviolet rays. The effect is a deep one, and indirectly through the circulation and is brought about by wavelengths that penetrate through the epidermis and part of the dermis. The surface action of ultraviolet wavelengths shorter than 290 $m\mu$ or of those slightly longer (up to 310 $m\mu$) in large quantity is detrimental to the healing of wounds, unfavorably influencing the processes of repair. The influence is due to the longer ultraviolet, luminous and infrared rays, and so the quartz mercury vapor lamp is not indicated, while natural sunshine and the energy from "Sunshine" carbons are.

Ultraviolet rays alone do not lower the blood pressure, but carbon arc radiation ("Sunshine" carbons) does—that is to say, of between 60 and 70 p.c. of persons with essential hypertension. In ambulatory and hospitalized patients systolic and diastolic pressure may be materially reduced (from 10 to 15 p.c.) by general carbon arc irradiation, given in erythema-producing doses, not too frequently, so as to avoid tanning. The "cure" is temporary, and the treatments have to be repeated, being given every ten days or two weeks in those whose pressures have been lowered by earlier and more frequent large doses (usually every fourth or fifth day).

Among diseases of the skin, lupus vulgaris is the only one on which ultraviolet rays act specifically. In some others it may have favorable action but the improvement is not specific. In others exposure to such rays may cause or provoke an attack.

Owing to the hypersensitivity of many infants and adults, caution should be exercised in the use

of sunlight and of artificial radiation. Over-indulgence, even in the normal, is foolhardy.

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DISCUSSION

Dr. Blum: Laurens suggests the relationship of a number of pathological light sensitivities in man to photodynamic sensitization, a subject which I shall discuss in detail in my paper on "Photosensitization of Living Systems." I would like at this time to present observations on a few cases of photosensitivity in man. We have studied four cases all showing different pictures.

- I. An erythematous dermatitis, not classified.
- II. Eczema solare.
- III. Urticaria solare.
- IV. Hydroa vacciniforme.

We examined these cases to determine the spectral radiation which produces these lesions. The methods were rough, involving the use of filters. To summarize briefly, in cases I and II we were able to reproduce the lesions by ultraviolet radiation shorter than 3200 Å, i.e.: those wavelengths

which produce erythema and pigmentation in man. Thus we can reasonably assume that these individuals exhibit some hypersensitiveness to the normal photoreactive mechanism of the skin. This may be a hyperactivity of the photomechanism or a hypersensitivity of the skin to the changes produced by the photoreaction. We have no reason here to suspect a photodynamic sensitization.

Case III presents a severe urticaria following exposure to radiations between 4000 and 5000 Å, i.e.: definitely outside the wavelength region producing normal erythema. Here we may suspect photodynamic sensitization. However, photodynamic sensitization only occurs when molecular oxygen is present and we have been able to show in this case that depriving the skin of oxygen does not prevent the development of the urticaria. (Blum, Allington and West, *J. Clin. Invest.*, July, 1935). On the other hand, urticarial reactions produced by sensitizing the skin with photodynamic dyes are completely prevented by the removal of oxygen. (Blum, Watrous and West, *Am. J. Physiol.* in press).

The case of Hydroa (IV) is a typical one displaying porphyrinuria. Unfortunately I have not been able to make all the observations I should like in this case, but on two occasions, exposure to sunlight for periods of 20 and 30 minutes has produced no result; this should cover the visible range. The patient has been receiving quartz mercury arc radiation over the whole body twice a week for about a year and has shown no tendency to develop lesions as a result of this. Obviously if the porphyrin is acting as a photodynamic sensitizer in this case, the patient should react to the radiations absorbed by this sensitizer. This does not seem to be the case and hence this condition cannot be categorically accepted as due to photodynamic sensitization.

It hardly needs to be said that the building of hypotheses such as those attributing all photosensitivity to disturbed sulphur metabolism, or to photodynamic sensitization, is not justified until we can classify these diseases according to the wavelengths producing them. Unfortunately very little attention has been given to such observations, particularly within recent years.

Dr. Singer: In this paper the role that pigmented cells play as protection against ultraviolet was discussed. It was also mentioned that other cells have a protective activity against ultraviolet radiation. It is quite difficult to say what we should consider pigment if we speak of radiations of certain wavelengths. It is better to say that we have in the organism filters with selective absorption. This denomination will include pigment cells that have color in visible light as well as cells that are transparent in visible light but have their absorption in the ultraviolet spectrum.

For example, I have observed in my experiments on the frog's skin that there is a system of fibers that is transparent to the visible rays but is opaque to the near ultraviolet. These fibers are situated in the layer which contains the skin glands and which lies directly below the epithelial layer. An exact study of these fibers shows them as lying directly above the elastic fibers and surrounded by the skin glands and blood vessels.

The frog's skin does not have corneated surface cells, therefore the ultraviolet radiation goes through the epithelial layer to the layer below which contains, as I mentioned before, the skin glands. It is probable that these fibers filter out parts of the radiation that would be harmful to the elastic fibers and therefore serve as a protective filter against ultraviolet radiation.

Dr. Abramson: It has recently been shown that histamine-like substances are formed in intact tissues after the use of different types of physiotherapy involving the skin. A plug saturated with saline was made the cathode and placed on the skin. At this pole, following treatment, concentration of the histamine-like substances occur in sufficient concentration to be assayed by a biological method.

Dr. Mayerson: I think the method presents many opportunities of studying substances in the skin, particularly since the skin is intact in the procedure.

Dr. Abramson: What is your point of view in regard to the efficacy of ultraviolet radiation in the therapy of erysipelas?

Dr. Mayerson: Some investigators have reported excellent results following irradiation in erysipelas while others have been severely critical of this method of treatment. However, one gets the distinct impression from the literature that the method is at least as successful as other accepted methods such as anti-toxin, roentgen ray irradiation, etc. and for this reason, if for none other, it should be more extensively employed and studied. One should be cautious, however, in ascribing the results obtained to the action of ultraviolet unless radiation of longer wavelengths, particularly infrared, has been eliminated.

Dr. Abramson: It seems strange that extrapulmonary tuberculosis is so strikingly and consistently benefited by radiation and that the pulmonary variety is not.

Dr. Meyer: Tryptophane and tyrosine, as aromatic compounds, have a marked absorption in the near ultraviolet while the aliphatic compounds absorb much shorter wavelengths. Have any analyses of the skin been made for these substances in cases of established sensitivity to ultraviolet radiation?

Dr. Mayerson: I do not know of any such

analyses. Harris and Hoyt found that a solution of tyrosine or aminobenzoic acid retarded the destruction of bacteria exposed to quartz mercury arc irradiation and concluded that the susceptibility of protoplasm to ultraviolet rays was conditioned by the selective absorption of the toxic rays by the aromatic amino acid radicals. It may be that in some of these cases there is a deficiency of aromatic compounds which accounts for their hypersensitivity.

Dr. Meyer: In cases of *sailor's skin* it would be interesting to estimate the sterols in the blood and skin to see whether these substances play any part in the production of the increased sensitivity to radiation.

A study of the absorption of histidine derivatives and substitution products would be desirable for the understanding of the production of histamine or histamine-like substances.

Dr. Hausmann: I agree entirely with the principles and views which Laurens expresses in his introduction. In connection with his discussion of Ellinger's work on the seasonal variation in erythema reaction, the question arose in my mind as to whether Ellinger's findings did not explain the contradiction which still exists between the physical studies of Dorno and the observations of some experienced heliotherapists. Dorno, as is well known, finds in the High Alps (Davos, Switzerland) that the intensity of solar and sky ultraviolet radiation is greatest in the summer, smallest in winter, only a little increased in the spring, while in the autumn it is greater than in the spring. But as Oscar Bernhard pointed out, in the high Alps the erythema reaction and the consequent pigmentation of the skin are particularly strong in the spring. I also have tried to study this problem. The investigations of Ellinger now partly explain the seasonal variation in erythema reaction and indicate that the increased sensitivity in March or April is probably due to increased thyroid activity. This helps to clear up the old contradiction.

Years ago I pointed out that the reflection of ultraviolet radiation from snow might be of importance in this connection, and this opinion has been confirmed by some of our own investigations. With F. M. Kuen, I studied the bactericidal action of ultraviolet rays reflected by snow, ice, water, stone, meadow-land and so on. We found that the bactericidal ultraviolet is most strongly reflected by snow and next by ice. The amounts reflected by chalk, granite, gravel and water, while still definite, are much less. Green meadow-land reflects the bactericidal rays the least. The reflection of ultraviolet radiation by snow, ice and water would seem to be of some hygienic importance (see Coblentz, Stair and Hogue).

In connection with Laurens' discussion of sensitization in the ultraviolet, my laboratory continues to be occupied with this question. Hausmann and Kuen have demonstrated sensitization in the ultraviolet to porphyrins. Desensitization in the ultraviolet by substances such as serum, glucose, levulose, pinakryptol and neosalvarsan was also observed. Na_2SO_3 increased the hemolytic action of the ultraviolet, particularly wavelengths 365-366 m μ .

Dr. Laurens: In connection with the marked influence of spring sunlight in the production of erythema and pigmentation in the high Alps and almost everywhere for that matter, it should be remembered that the persons so acted on have for the most part been protected from the sun's rays during the preceding winter and they have naturally become more sensitive to the solar ultraviolet by being unaccustomed to it as a result of lack of exposure. Furthermore, the content of spring sunlight in ultraviolet, although comparatively low, is by no means inconsiderable. I have discussed this topic on pp. 36 and 112 of my book: *Physiological Effects of Radiant Energy*.

The use of a salt-free diet (the so-called Sauerbruch-Herrmannsdorfer-Gerson diet) has not become popular outside of Germany and Austria. It is frequently combined with radiant energy treatment, and even with douche baths, for the thorough cleansing of the skin. At the Finsen Institute the diet is rich and adequate and not restricted in any way. As everyone generally acquainted with the subject knows, there are numerous claims of outstanding success in the treatment of tuberculosis of the skin by various methods, e.g., by so-called "Grenz rays", (extremely long and soft X-rays 1 to 4 Angstroms) either alone or combined with a salt-free diet and with ultraviolet rays. The English believe the salt-free diet to be not only trying to the patient but of no benefit.

Dr. Ellinger: Right at the beginning Laurens states that the number of light-healings claimed and those actually proven, show a vast disproportion, a statement with which I thoroughly agree. He shows what an enormous quantity of material pertaining to ultra-violet light treatment has been gathered in a comparatively short period, so as to make available today a rational "Light-Therapy" with distinct indications and counter-indications. This fact was so clearly shown that it would only lessen the deep impression made upon all hearers, if I should go into detail once more as to the main points.

1) In a number of cases mentioned by Laurens such as pregnancy, menstruation, vegetative stigmatism, thyreotoxicosis, certain forms of eczema, tuberculosis, etc., a "light-biological" examination has shown a heightening of the sensi-

tivity of the skin. It is interesting, therefore, to recall to your mind that this heightening of sensitivity is noticeable in the very same cases under X-ray treatment and therefore, in my opinion, it is not unlikely that one may find in this biological light-research a most practical method for the prevention of damage by X-rays.

2) I have shown in former researches* that a parallel exists between the sensitivity under the light of a mercury vapor lamp and under sunlight, in spite of their different spectral compositions. On the basis of these findings I was able to elaborate a method for determining the value of protective measures against light.

3) I should like to call attention once more to the relations, mentioned by Laurens, between the light-sensitivity and the number of functioning skin-capillaries on one hand, and the influence of the "thyroid gland" upon these capillaries on the other hand. Laurens shows us the heightening of the sensitivity under light in the case of "Thyreotoxicosis" as well as in "Vegetative Stigmatism". We see how, in the case of pregnancy, this fact is projected upon the skin by way of the hormone-producing glands. Again and again we are confronted with the fact that the thyroid gland holds a central position and always seems to play its part in hormonal changes, and it is in my opinion this gland, to which must be attributed a regulating influence of ray-reactions upon the skin. Whether in all these functions the hypophysis is influencing, in its turn, the thyroid, as has been shown in many other instances, is a matter of conjecture, though it appears most probable after observations made in light-sensitivity during pregnancy. The supposition that the thyroid is partly responsible in the heightening of the sensitivity under light in the case of pregnancy and that its enlargement is also a primal cause of the enlargement of the frontal lobe of the hypophysis, and in connection therewith an increased formation of thyreotropic hormone seems to be justified on the basis of our present experiences. Furthermore, the heightening of the sensitivity under rays during the springtime undoubtedly is due to the activity of the thyroid. This was clearly shown on the chart and the respective curves presented by Laurens. An increased activity of this organ has been shown by several other scientists to be in evidence during the springtime, as reflected in changes of the iodine within the blood. This heightening of the sensitivity under light is not in evidence with human beings alone, but with rabbits as well. This condition, however, is not to be found if the animals are thyroidectomized in time, as I have shown in former treatises,

* F. Ellinger: *The Biological Principles of "Ray-Treatment"*. Berlin 1935. Urban and Schwarzenberg.

while on the other hand this sensitivity can be heightened through thyroxin poisoning.

4) Laurens has pointed out the fact that the ultraviolet rays form histamin and thus resemble ingredients affecting the skin in a similar manner. Histamin, it may be stated, can be considered a capillary poison. If, therefore, we consider this fact in connection with the findings just stated, as regards the influence the thyroid is exerting over the functions of the capillaries of the skin, we perceive the fact that the skin itself is a mediatory channel between the inner and the outer world, i.e. the sensitivity of the outer sphere influences, by way of the histamin, the inner body, while the thyroid gland, as the main spring for inner conditions, is the cause of the sensitivity of the skin under effects from the outer sphere.

I shall be glad if I have succeeded in showing in these few remarks how complex is the variety of problems of clinical, general biological and technical nature, as shown in Laurens' lecture.

Dr. Harris: Referring to the increased incidence of gastric ulcers in the spring and fall, Laurens states, "That the incidence of ulcers does not increase in the summer when the ultraviolet intensity is high is due to the hot weather, which diminishes the secretion of gastric juice." I was much interested, during a recent visit to South America, to learn from the chief surgeon of the United Fruit Company hospital at Santa Marta, Colombia, that gastric ulcer is unknown in that region. Santa Marta is at sea-level, not far from the equator, and hot at all seasons of the year. The facts coincide admirably with Laurens' statement, though other factors may also be involved.

EFFECTS OF RADIATION ON CIRCULATION, BLOOD AND METABOLISM, WITH PARTICULAR REFERENCE TO GROWTH, BASAL METABOLISM AND THE THYROID GLAND

H. S. MAVERSON

The wide spread interest manifested during the last two decades in the effects of radiation was incited in large measure by the findings in connection with rickets. A considerable literature has accumulated dealing with the part played by radiation in the regulation of various physiological functions and in the treatment of different diseases. In much of the work there has been a lack of control and specification of experimental conditions which makes it difficult to analyze and correlate the results of different investigators. Excepting rickets, outstanding results have been obtained only in extrapulmonary tuberculosis. On the other hand, there are definite indications that radiation, under certain circumstances and when combined with other factors such as diet and hygiene, may exert an effective auxiliary influence on physiological and pathological processes. The proper evaluation of the part played by radiation as an adjuvant in many reactions may eventually help to explain its mode of action.

The literature dealing with the physiological effects of radiation has recently been detailed by Laurens (1). It would be impossible to discuss any phase of this subject without drawing heavily on this splendid monograph. It has served as the source of much that appears in this review and for this invaluable aid the writer is gratefully appreciative.

EFFECTS OF RADIATION ON THE CIRCULATORY SYSTEM¹

BLOOD PRESSURE AND PULSE RATE

In 1905, Hasselbalch (2) and later Hasselbalch and Jacobaus (3) described the effects of radiation on blood pressure. Massive irradiations with a powerful carbon arc, given at such intervals as to maintain a lasting hyperemia with avoidance of pigmentation, resulted in decreases in blood pressure averaging about 10 p. c. and lasting for several months. Marked relief of pain and freedom from attack followed the irradiation of patients with angina pectoris. In explanation of the results, the authors suggested that the redistribution of blood resulting from the cutaneous hyperemia and the subsequent lowering of peripheral resistance in the smaller vessels was reflected in a decreased pressure in the large vessels and mechanical relief to the heart. No

significant or definite influence on the pulse rate was noted.

Subsequent clinical and experimental work (1, chapter 6) has confirmed and amplified these results. At the present time there is general agreement that irradiation with the carbon arc will produce a decrease in blood pressure which persists for longer or shorter intervals depending on the intensity of the exposure. Mercury vapor arc radiation seems to be relatively less effective in this regard, while solar radiation produces only slight changes. Pulse rate effects are inconstant and may vary in either direction, or show no change.

The typical changes in the blood pressure and pulse rate following a single moderate exposure of a normal dog to carbon arc radiation² are given in Fig. 1. The systolic pressure decreases

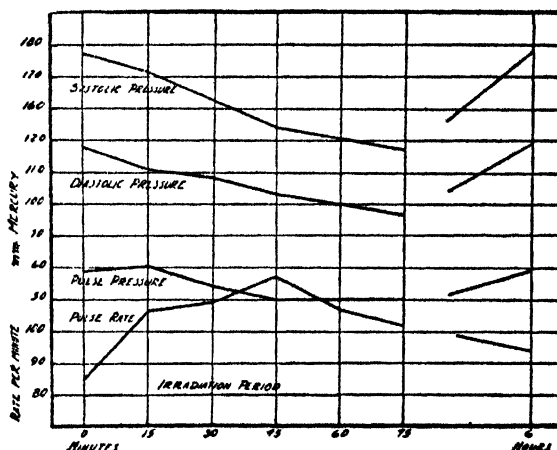


FIGURE 1.

Abdominal carbon arc irradiation of dog for 1 hour at 40 cm. (55 g. cal. per sq. cm.). (Laurens and Mayerson (4)).

from 5 to 20 p. c. in different cases, remains low for about 5 hours and then shows a gradual return to normal. The diastolic pressure in some cases shows a proportional decrease; in others,

¹ The terms "radiation" and "radiant energy" as used in this paper include only those wavelengths usually present in solar radiation.

² The carbon arc used in most of the work in our laboratory is a "Pan Ray Arc" made by the Atlas Electric Devices Co. National Carbon Co. Therapeutic A ("Sunshine") carbons are usually used. The arc operates at 25 to 28 amp. and 55 to 60 volts, and emits a total energy averaging about 0.5 g. cal. per sq. cm. per min. at 1 M., distributed approximately as follows: 4 p. c. < 400 mμ 31 p. c. 400 mμ to 1.4μ; 65 p. c. > 1.4μ.

it increases during irradiation and returns to normal soon after. The pulse rate usually increases during the exposure, but this effect is not uniform. If massive, repeated exposures are given it is possible to produce persistent low levels. Thus in two dogs used in the above study, massive irradiation (83 to 124 g. cal. per sq. cm.) at intervals of 2, 3 and 6 days resulted in a depression of systolic pressure which was maintained for 14 and 20 days, respectively, with values 12 and 22 p. c. below normal, and the diastolic for 15 and 10 days with values 12 and 21 p. c. below normal. The pulse rate increases, noted in these experiments, were interpreted as being attempts to compensate for the lowered blood pressure and to maintain an adequate cardiac output.

These results suggested to Laurens (1, p. 172) the use of carbon arc radiation in the treatment of human hypertension. Exposures intense enough to set up inflammatory reactions and marked vasodilatation were used and repeated at such intervals as to avoid pigmentation. Average decreases of 10 to 15 p. c. in the systolic and diastolic pressures were obtained in about 60 to 70 p. c. of the cases studied. By continuing the irradiation at intervals of 10 days to 2 weeks, it was possible to maintain these low levels for long periods (Fig. 2). Pulse rate changes were variable and insignificant.

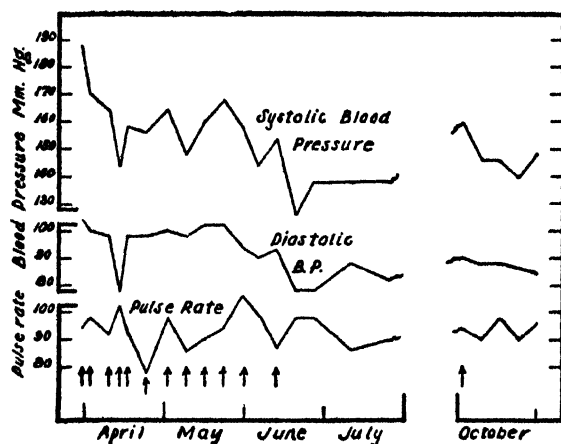


FIGURE 2.

Effects of carbon arc radiation in hypertension. Arrows denote exposures. (Subject A, Laurens (1))

Various explanations of the mode of action of radiation in lowering blood pressure have been suggested. Kestner and his collaborators (5) ascribed the effect not to direct action of the radiation but to the inhalation of combustion products, such as ozone, nitrous oxide, etc., emitted by the arc, but this has not been confirmed (4).

Rothman (6) does not subscribe to Hasselbalch's explanation of cutaneous hyperemia, but believes that the lowering of blood pressure is due to a decrease in sympathetic tone which manifests itself in addition by a marked drop in blood sugar, increased sugar tolerance, decreased adrenalin secretion, and a relative lymphocytosis and eosinophilia. Petersen and Ottingen (7) have elaborated this point of view. They concluded that irradiation of the skin resulted in a disturbance of the balance between peripheral sympathetic and splanchnic parasympathetic tone, so that the therapeutic action following radiation consisted of an overbalance of the parasympathetic tone in the splanchnic area, with the consequent changes described above.

The work of Lewis (8) concerning the reactions of cutaneous vessels to tissue injury by freezing, burning and the action of ultraviolet, suggests another plausible explanation of the mode of action of radiation in lowering the blood pressure. As a result of tissue injury there are liberated into the surrounding skin substances with histamine-like action leading to the "triple response", a local dilatation, a reflex dilatation and increased permeability of the minute vessels. The drop in blood pressure, following radiation, may conceivably be due to the action of these vasodilating substances carried in the peripheral blood stream to the rest of the body. The experiments of Harris (9) support such an explanation. He showed that irradiation of the back resulted in an increase in the volume of the forearm as measured by the plethysmographic method. The effect would be enhanced by the improved blood flow resulting from the vasodilatation which usually accompanies irradiation, particularly when erythema-producing intensities are used. (See Ellinger's work, discussed by Laurens in preceding paper.) The more marked lowering of blood pressure obtained with carbon arc than with quartz mercury arc radiation is to be explained, on this basis, by the more extensive hyperemia which the former induces. The carbon arc not only emits ultraviolet and luminous, but a considerable amount of penetrating red and infrared, radiation. It produces, therefore, not only an ultraviolet erythema affecting the vessels of the superficial skin layer but a heat erythema as well, which involves the vessels of the deeper layers (see Laurens, 1, pp. 120 and 183). If the effect of radiation in lowering blood pressure is specifically due to the action of the short ultraviolet wavelengths in producing local tissue changes, this effect must be considerably reinforced by the increase in blood flow. There is no direct evidence, however, at the present time which implicates any particular part of the spectrum in this effect.

CARDIAC OUTPUT

Lindhard (10), in 1913, irradiated seven subjects with the carbon arc and found the cardiac output increased 10 to 30 p. c. in four cases, and decreased in the remainder. He concluded that these variable results were due to differences in the compensatory mechanisms of the different subjects to the cutaneous vasodilatation.

Pollock, Laurens and Johnson (11) and Johnson and Laurens (12) have recently studied the effects of carbon arc radiation on the cardiac output of normal dogs and normal and hypertensive men. The results on dogs were consistent (with one exception) in showing decreases in cardiac output of from 21 to 29 p. c. roughly paralleling the lowering of the blood pressure. The maximal changes occurred 5 to 43 hours after irradiation, with a return to normal by the fourth day. Oxygen consumption showed insignificant changes while oxygen utilization increased. On the other hand, in the experiments on humans, cardiac output showed a predominant tendency to increase. Thus in eight normal subjects irradiated in the basal state with erythema-producing exposures there was a significant tendency toward a slightly decreased blood pressure, a variable effect on pulse rate, no change in oxygen consumption, but a markedly increased cardiac output of 12 to 35 p. c., reaching a peak on the second or third day after irradiation and returning to normal by the fifth or sixth day. The results with the hypertensives, twelve subjects with systolic pressures varying from 191 to 228 mm. Hg, were very much the same, except that the increases in cardiac output were usually greater and appeared sooner, usually reaching a maximum on the first day after radiation (Fig. 3). Blood volume, which was studied in a number of cases, showed increases paralleling those in cardiac output.

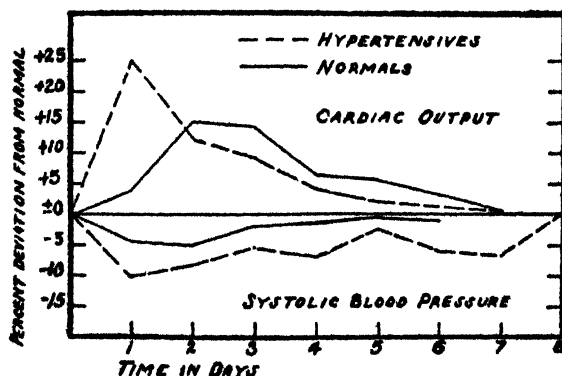


FIGURE 3.

Effects of single carbon arc exposures on cardiac output and blood pressure. (Johnson and Laurens (12)).

The differences in the effects on the dog and on the human are at present difficult to reconcile. The changes in the dog, a decrease in cardiac output concomitant with a drop in blood pressure may be due to a condition approaching shock, brought about by extensive histamine production and consequent vasodilatation. Johnson and Laurens suggest that the dog may be more sensitive to histamine than the human, which may also explain the difficulty of lowering blood pressure in the normal human as compared with the normal dog. They also tend to feel that the increase in cardiac output observed in the normal and hypertensive patients may be a compensatory reaction secondary to the vasodilatation and drop in blood pressure, and suggest that the failure of the output to increase may be an indication of impaired regulatory mechanisms.

EFFECTS OF RADIATION ON THE BLOOD

EFFECTS OF DARKNESS

Much has been written about the pallor of persons working or living in poorly lighted rooms, or in polar regions, etc., but as Laurens (1, chapter 7) points out, this pallor is usually not accompanied by any decrease in the absolute number of red cells or in the concentration of hemoglobin, but is the result of a decrease in the volume of blood going to the skin and underlying muscles due to a vasoconstriction of peripheral vessels. Such "anemic" looking people, in spite of their pallor, often have a surprisingly high hemoglobin content. Studies made of men on polar expeditions fail to show any effects of the long polar night. Borchardt (13), in a more recent study, compared the hemoglobin values of a large number of women and children in northern Norway and in Hamburg and came to the conclusion that the former were suffering from a mild polar anemia during the winter season. Quartz mercury arc irradiation of these "anemic" children, however, did not improve the hemoglobin level, and a subsequent examination of the same population in the summer showed only slightly higher hemoglobin levels. It would seem that other factors, nutritional or otherwise, were responsible for the observed anemia, rather than the lack of radiation. Studies made on mice, rats, dogs, mules and horses, kept in darkness or dim light for considerable periods of time, fail to indicate that absence of radiation produces more than slight or transient changes in the blood picture. These are not significantly affected by subsequent irradiation (1, chapter 7).

EFFECTS OF RADIATION

Any action of radiation on blood must take place chiefly through the blood in the superficial

capillaries. As Laurens has pointed out in the preceding discussion, the layer of capillaries in the epidermis absorbs ultraviolet and a part of the visible rays at slight depths, the absorption curve rising from the long waves to the short with a maximum between 415 and 400 $m\mu$. The red cells coursing in the capillaries absorb about 5 p. c. of wavelength 680 $m\mu$, 92 p. c. of 405 $m\mu$, and 75 p. c. of 290 $m\mu$. The greater the hyperemia, i. e., "the more the capillaries are filled so much the more will the shorter rays be taken up by the blood and given to the organism as a whole, and so much the more the long waved energy will pass through the network of blood vessels and penetrate to the deeper layers of the body." It is therefore essential that the quality and quantity of radiation be accurately specified in order that results of different investigations may be properly interpreted or duplicated. A source of dosage which produces a marked hyperemia might be expected to give results differing from those under conditions where no hyperemia was obtained.

This is particularly true since changes in blood volume also usually accompany the hyperemia. Barkus and Balderrey (14) showed that carbon arc irradiation of sheep was accompanied by a vasodilatation followed by diffusion of tissue fluid into the blood stream, causing blood dilution and increased blood volume. With long exposures, the dilution was counteracted by overheating, resulting in vasoconstriction and blood concentration. These findings were confirmed by Mayerson (15) and Mayerson and Laurens (16) on the dog. The latter found that the primary result of an individual exposure to carbon arc radiation is a temporary increase in plasma volume of 6 to 37 p. c. with recovery to normal within 5 hours. They also found that with massive exposures a slight concentration follows the initial dilution. Many of the effects described as resulting from radiation, particularly those during or immediately after radiation, can unquestionably be explained on the basis of changes in blood volume.

The results obtained on irradiation of experimental animals and men with artificial sources (chiefly carbon and quartz mercury vapor arcs) indicate very little effect on the red blood cell count and hemoglobin content, when these levels are normal at the beginning of the investigations (1, chapter 7). Strong, or long continued, irradiation of individuals with low initial levels results in most cases in a slight rise (seldom averaging more than 10 p. c.) in the erythrocyte count, and a concomitant, but smaller, increase in hemoglobin which is maintained for a longer or shorter time after the irradiation is discontinued. This is particularly true in the various forms of tuberculosis, where the results have been more

constant and marked, radiation raising the initial low red blood cell and hemoglobin levels to normal. It is difficult to decide, in many of these cases, as to how much of the benefit is primary, or secondary to the general bodily improvement. The results of Ellinger (17a) are of interest in this connection. He found that active cases of tuberculosis, as judged by their erythematous responses to radiation, were much more sensitive than normal healthy individuals. He also showed (17b) that increased sensitivity was usually associated with a greater number of open capillaries. It would be interesting to have experiments in which the erythematous sensitivity, number of open capillaries, blood cell and hemoglobin responses were followed in the same tuberculous.

While the total leucocyte level does not seem to be influenced significantly or consistently, there is a general agreement of data showing that exposure to sunlight or artificial radiation results in a definite lymphocytosis. Hardy (18) carefully studied the changes in the number and distribution of white blood cells in rabbits exposed to measured amounts of radiation from a mercury vapor lamp. By the use of filters and by varying the lengths of exposure, she obtained equivalent amounts of ultraviolet radiation, but in different spectral regions, as well as different amounts of total energy in the same spectral regions. Radiation of wavelengths shorter than 300 $m\mu$ resulted in a lymphocytosis, providing the dose was not too great, the increase reaching a maximum 2 to 3 days after the irradiation. Hardy believes that the effect of ultraviolet radiation on the lymphocytes is not selective, but is proportional to the total amount of far ultraviolet radiation present, regardless of wavelength. Irradiation with wavelengths 275 to 320 $m\mu$ gave a sharp and persistent increase in polymorphs, which effect was selective and proportional to the energy in this region. Since the amount of energy producing a rise in polymorphs is greater than necessary to produce a lymphocytosis, the two effects are not observed simultaneously.

Relatively little attention has been paid to the effects of radiation on the platelet count. Where they have been studied (16, 18a, 19-25) the results are consistent in demonstrating in animals and men a definite and sustained rise in their number. This effect suggested to Sooy and Moise (26) the use of quartz mercury vapor lamp radiation in idiopathic purpura hemorrhagica in which thrombocytopenia is characteristic. They reported hematological and clinical improvement in the ten cases, five acute and five chronic, which they studied. The author (27) has treated one case of the disease in a 5 year old boy who had proved refractory to all forms of treatment and for whom splenectomy had been advised.

Seven graded carbon arc irradiations over a period of 2 weeks increased the platelet count gradually from 45,000 to 400,000, with complete clinical recovery. The platelet count remained at this level for 3 months after the irradiation was discontinued, at which time the patient was dismissed. The boy has been symptom-free for the four years which have elapsed since the treatment. In contrast to these favorable results, Tolstoi (28) was unable to find any improvement in three chronic cases which he irradiated with the quartz mercury vapor lamp, and warned against too hastily accepting a treatment for a disease in which spontaneous recoveries often occur. The etiology of purpura, however, is still in question, and the usual forms of therapy, hygiene, diet, X-ray radiation, transfusion and splenectomy are not always successful. It would seem that the simple and rational method of radiation should be given a more extensive clinical trial to test its specific or adjuvant effects in this disease, before being dismissed as ineffective.

Figure 4 illustrates typical responses following individual irradiations in the dog as obtained in our laboratory (16). The resultant blood dilu-

repeated massive carbon arc irradiation. In five such experiments on dogs there was an increase in red cell number of from 10 to 19 p. c., maintained for from 3 to 6 weeks after the last irradiation. The changes in hemoglobin were slight (average increases 2 p. c.) and the color, volume and saturation indices showed that the cells in the post-irradiation period were usually smaller and less saturated than normal. Effects such as these have frequently been observed in conditions where there is a strong stimulus to erythropoiesis, small pale cells in regeneration from hemorrhagic anemia (29, 30), and in emotional polycythemia (31). In two experiments platelet levels were increased 4 and 15 p. c., respectively, and remained high for about 10 days after irradiation was stopped. Most of the animals showed a progressive leucopenia, resulting in low post-irradiation leucocyte levels.

The results outlined thus far have been obtained, for the most part, on animals and men whose red blood cell and hemoglobin levels have been normal, or only slightly decreased. Since the normal mechanism is relatively stable, it seemed plausible to expect that radiation would

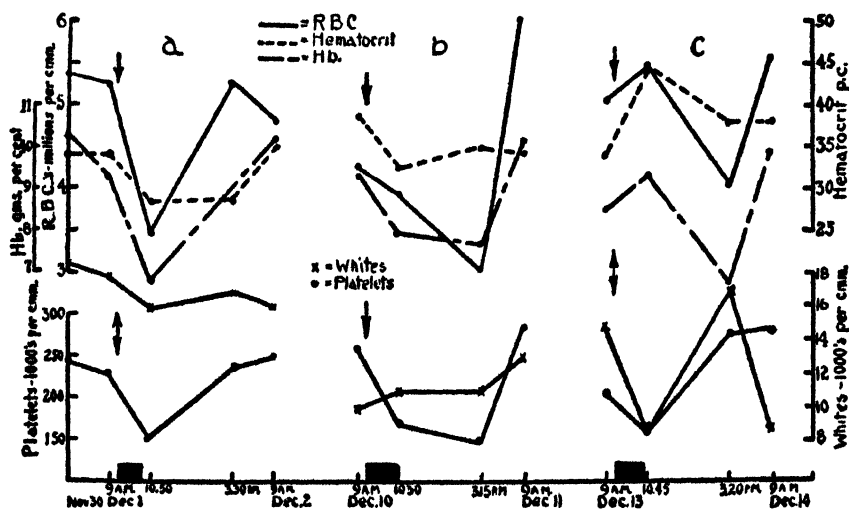


FIGURE 4.

Typical effects following carbon arc radiation. Arrows and heavy blocks show beginning and approximate duration of exposures. a. First irradiation, $\frac{1}{2}$ hr. at 1 M. (36 g. cal. per sq. cm.). The first points indicate average pre-irradiation levels. b. Tenth daily irradiation, 1 hr. at 60 cm. (133 g. cal. per sq. cm.). c. Thirteenth daily exposure, dose same as in b. (Mayerson and Laurens (16)).

tion is best indicated by the changes in the platelet count. It occurs with each successive exposure, but is not augmented by the exposures. The duration of the dilution is, however, determined by the dosage and interval between successive exposures. Figure 5 portrays the principle blood changes in a typical case during and after

provide a more striking stimulus to hematopoiesis in anemic conditions. Figure 6 shows the results of carbon arc irradiation in hemorrhagic anemia (32). These are typical curves obtained in a study of twenty-four dogs, in whom a severe secondary anemia was produced following the procedure of Whipple and Robscheit-Robbins

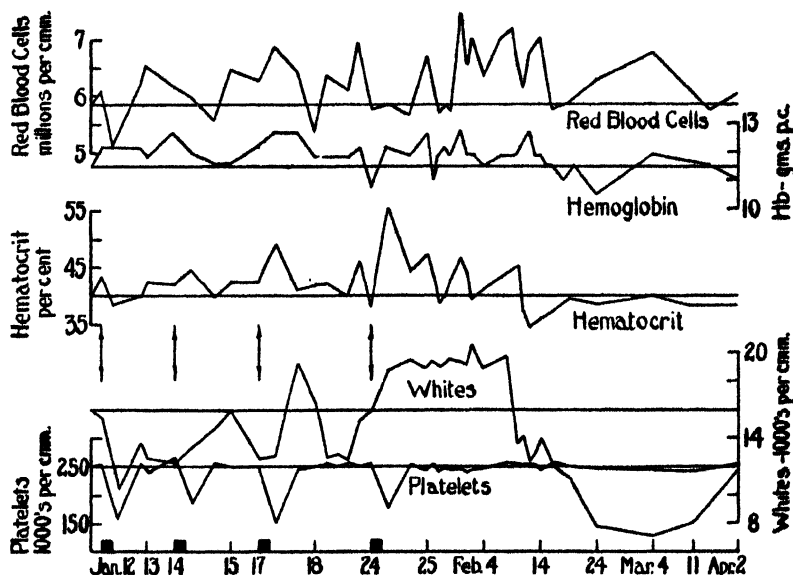


FIGURE 5. Arrows and heavy blocks show beginning and approximate duration of exposures. First 3 exposures are $\frac{1}{2}$ hr. at 60 cm. (67.0 g. cal. per sq. cm.). Fourth exposure is $\frac{1}{4}$ hr. at 60 cm. (100 g. cal. per sq. cm.). Continuous horizontal lines show average pre-irradiation levels. (Mayerson and Laurens (16)).

(29). The animals, fed a standard diet designed to minimize hemoglobin production, were made anemic by bleeding, and the hemoglobin was kept at a constant low level by periodic withdrawal of blood, the efficacy of the treatment being measured by the amounts of blood (expressed as grams of hemoglobin) which it was necessary to remove in order to maintain the low level. Carbon and mercury arc radiation resulted in marked and persistent increases in the number of erythrocytes and reticulocytes, but in only one experiment was there any increase in hemoglobin regeneration. Radiation also failed to materially enhance the effects of moderately potent stimulants to hemoglobin formation, such as peaches, lettuce and apricots, which were added to the diet in some experiments. These results are interpreted as indicating an inability of radiation to substitute for dietary deficiencies in regard to the formation of hemoglobin, a point which has been emphasized in studies in rickets, in which it has been clearly demonstrated that radiation is effective only by virtue of the fact that it enables or permits the organism to utilize more economically materials present but not available. The marked responses of the red blood cells in the absence of any definite hemoglobin regeneration suggested that radiation acts on the mechanism for the production of stroma, rather than on that by which hemoglobin is made available.

The experiments outlined above were a very severe test of the efficiency of radiation. Not only was hemoglobin removed from the body, but the diet furnished a bare minimum of material which

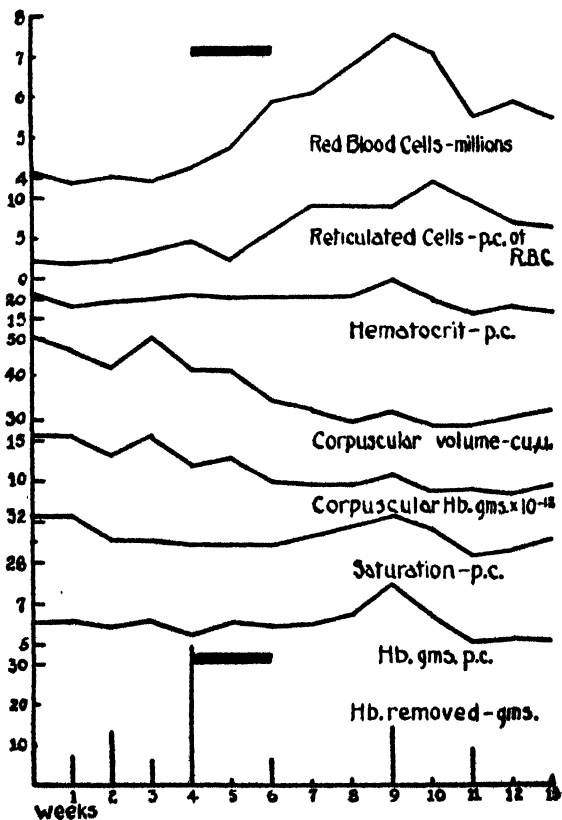


FIGURE 6.

Carbon arc radiation of dog for 60 minutes at 80 cm. every other day for a total of 8 exposures. Heavy blocks denote the period of irradiation. (Laurens and Mayerson (32)).

could be used in the manufacture of new hemoglobin. A less severe, and perhaps more favorable, condition was furnished in studies on a hemolytic anemia, produced by the injection of acetylphenylhydrazine in dogs (33). In this type of secondary anemia there is little loss of hemoglobin from the body, and the retained hemoglobin may be utilized in the formation and maturation of new and functional red blood cells. Twenty-four dogs were used, five being irradiated with the quartz mercury vapor arc, eight with the carbon arc, and the remainder serving as controls. The results are briefly summarized in Figure 7. No significant differences in the rate

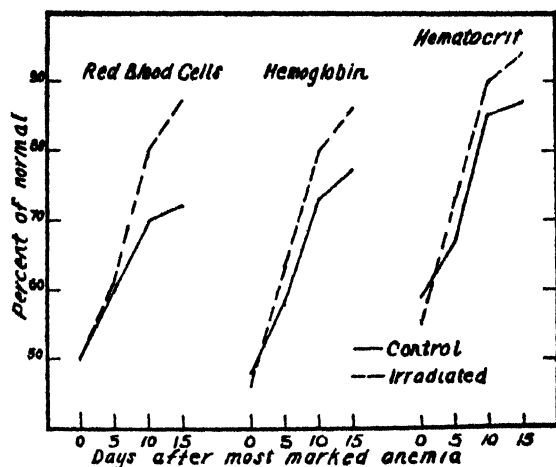


FIGURE 7.

Influence of carbon arc radiation on recovery from hemolytic anemia in the dog. (Mayerson and Laurens (33)).

of development of the anemia were observed in the irradiated and non-irradiated animals, but the regeneration was unquestionably faster in the irradiated group. The carbon and mercury arcs were equally effective, the average daily gain in red blood cells for 15 days in the latter group being 142,000 as compared with 101,833 for the control group. Similarly, the average daily hemoglobin gain for 15 days was 0.31 gm. in the irradiated, as against 0.23 gm. in the control group.

Our failure to obtain active regeneration of hemoglobin in the studies on hemorrhagic anemia, and our success in these experiments on hemolytic anemia, are, as indicated above, due to the difference in the experimental procedures. Osato and Tanaka (34) concluded that following irradiation the reserve iron is withdrawn from the organs and is used in the manufacture of hemoglobin. The better regeneration following irradiation in hemolytic anemia may be due to a more efficient utilization of the iron made available by the destruction of hemoglobin. The action

of radiation on the pigments set free may also be an important factor.

There has been relatively little work done in determining the effectiveness of radiation in nutritional anemia. Some investigators (35) have obtained beneficial results in exposing anemic pigs to sunlight, while others report no significant changes (36, 37). Baumann (38) in 1926 irradiated fifty-four children, fed exclusively on a milk diet for from 3 to 8 weeks. All the specifically rachitic symptoms disappeared, but the anemia remained. The anemia could be prevented by the addition of vegetables and fruit to the diet, or by giving reduced iron. Beard and his collaborators (39, 40) have recently reported that radiation markedly enhanced the effects of iron in bringing about recovery from the anemia produced in rats by the feeding of milk, while radiation was much less effective in the absence of iron. In preventive experiments the best responses were obtained when irradiated milk and iron were fed to the rats, suggesting that the observed beneficial effects of radiation were due to the formation of vitamin D in the animals and in the milk. Addition of vitamin D to the diet of rats fed milk and iron gave results similar to those of radiation and iron. These results present interesting problems for future study as to the relationship of vitamin D, calcium and phosphorus and iron metabolism.

The results of the clinical studies on the effects of radiation in secondary anemia are, in general, consistent with experimental studies. It is generally admitted that pernicious anemia is not materially improved by radiant energy, (see 1, p. 195), although Smith (41) believes that in the United States there is a relationship between deficiency in radiation and mortality from pernicious anemia. The improvement in the mild secondary anemia of tuberculosis and rickets has already been indicated. In secondary anemia associated with other conditions, the results have not been as regular. Thus Perlman (42) treated seventy-two children from 2 to 13 years of age with the quartz mercury vapor lamp. All showed some degree of anemia, coincident with chronic bronchitis, under-nutrition, etc. There was definite improvement in the erythrocyte level in forty-five cases, a decrease in twenty-six, and no change in one case. The hemoglobin level was increased in forty cases, decreased in twenty-eight cases and was not changed in four cases. One of the chief reasons for the variation in the effect of radiation in these clinical studies is the failure to recognize that radiation in itself is not specific but adjuvant in its action. Feeding of a diet rich in substances favoring hemoglobin production, in addition to the radiation of the pati-

ents, might conceivably result in improvement in a larger number of cases.

Our discussion so far has dealt with the effects on the blood, following irradiation through the skin. In 1922, Naswitis (43) inserted a quartz tube in the carotid or femoral arteries of dogs and irradiated the blood directly with a quartz mercury vapor lamp. He obtained marked and persistent increases in the erythrocyte count. Seyderhelm in 1932 (44) confirmed and extended these observations in a study of hemolytic secondary anemia, produced in dogs by the injection of saponin. Direct irradiation of the blood *in vivo* checked the development of the anemia even when saponin infusions were continued. The beneficial action was not due to a detoxication of the saponin but to an outpouring of young erythrocytes into the blood stream. Similar results were obtained when blood was removed, irradiated *in vitro*, and reintroduced into the animal. Seyderhelm believed that the active anti-anemic principle, which he called "Cytagenin" was associated with the stroma, and succeeded in separating it in a clear, protein-free solution. Clinical tests of the substance showed significant hemoglobin regeneration and good subjective improvement in the hypochromic anemia due to carcinoma and pulmonary tuberculosis, while larger doses were necessary in chronic septic conditions and in pernicious anemia. The responses in the latter condition were definitely poorer than with liver extract. Fervers (45a) has also reported a similar study. *In vitro* irradiation of citrated blood resulted in the production of an active anti-anemic substance which was much more effective in normal and anemic humans than was simple autohemotherapy. The effect was unusually rapid, the increase in erythrocytes and hemoglobin appearing in about half an hour after injection and reaching a maximum in 2 to 3 hours, which was maintained for 1 or 2 days. Repeated injections always increased the levels again. In one case of hemorrhagic anemia the red blood cell count rose from 1.4 to 4.8 millions after three injections, given over a period of 2 weeks. The spleen is in some way concerned with the action, since there was no improvement in splenectomized human cases. In addition to the beneficial results in hemorrhagic anemia, successful results were also reported in lymphatic leukemia, pernicious anemia, and the anemias associated with exophthalmic goiter and asthma. Fervers (45b) also reported the separation of the active principle "Ultragenin" from fresh irradiated animal blood in the form of a dry extract which can be taken by mouth. A similar extract but from non-irradiated blood failed to show any anti-anemic activity. Seyderhelm and Fervers believe that radiation activates a precursor

present in the red blood corpuscles (Seyderhelm believes it is in the stroma), resulting in the formation of the active anti-anemic substance, much in the same manner as vitamin D is formed from the provitamin by the action of short ultraviolet wavelengths. The wavelengths responsible for the activation of the precursor of the anti-anemic substance are, however, not known, and details are still lacking as to the methods of preparation, stability, relative effectiveness when compared with other anti-anemic treatments, etc.

EFFECTS OF RADIATION ON METABOLISM

From time to time there have appeared reports of cycles or seasonal variations in growth, basal metabolic rate, and in thyroid size and iodine content. The temptation has been strong to ascribe to radiant energy, particularly to ultraviolet radiation, a major role in the production of these cycles, disregarding the action of numerous other factors, such as humidity, temperature, diet, activity, etc. A survey of the literature dealing with the specific effects of radiant energy on growth, basal metabolism and thyroid activity reveals that relatively little well controlled work has been done on the human, while the results obtained on lower animals are confusing and contradictory. For the most part, however, the evidence tends to indicate that radiation *per se* has an insignificant effect on these functions.

GROWTH

Nylin (46) studied the growth of a large number of Swedish pre-school and school children and found periodic variations during the year. The height increase was pronounced during the summer and showed two maxima; a marked maximum during March and April and a smaller but distinct maximum during November and December. The minimum height increases occurred during September and October and January and February. In general, weight increase varied inversely with height increase. Two groups of twenty-five boys each were irradiated at different times for periods of 57 to 70 days (total irradiation of about 7 hours) with Jesionek quartz mercury vapor lamps supplemented with long waved radiation from "Sollux" lamps. The average increases in height of the irradiated groups, only slightly greater than those of the controls (0.3 to 0.4 cm.), were considered to be significant and were ascribed to ultraviolet radiation, even though the children were also exposed to long wave energy. Nylin attempted to correlate the periodicities in growth with the known seasonal variations in ultraviolet radiation. The spring maximum thus coincided with the increase in solar ultraviolet radiation which usually occurs in March, but it proved difficult on this basis to

account for the distinct maximum in December. Nylin naively dismisses this as follows: "Regularly recurring winter maxima in height increase in my investigations (for which other explanations are conceivable) speak against the assumption of an intimate connection between sunlight and growth." No evidence was obtained in Nylin's studies that the growth of children in "dark" schoolrooms differed from that of children in "light" rooms.

Studies on lower animals have, as a general rule, shown that radiation has only a negligible influence on the growth and development of mammals fed adequate and complete diets. All results are consistent in showing that rats reared on an optimal diet grow as well in darkness as in well-lighted rooms, and that irradiation with the quartz mercury vapor lamp has no demonstrable effect unless massive exposures are used, in which case there may be an inhibition of growth (1, chapter 11; also (47)). The author has recently confirmed these findings in a study involving 190 albino rats (48). The details of these experiments will be given later in the discussion on the relationship of radiant energy and the thyroid gland. There was no indication that darkness *per se* (even in rats born and kept for 16 weeks in darkness) interfered with normal growth; on the contrary, some of these rats had the largest terminal weights of the entire group. Nor did irradiation with the quartz mercury vapor lamp, carbon arc or sunlight improve growth.

Special mention should be made of the pioneer work of Hume (49) and of Goldblatt and Soames (50) in investigating the effect of radiant energy on rats suffering from vitamin A deficiency. This work, carried out before the separate identity of vitamin D was well established, led to erroneous interpretations as to the relationship between radiant energy, growth and the vitamins, but as Luce-Clausen (51) points out, the work served as the starting-point of important investigations for other workers. They found that while irradiation did not protect the animals from xerophthalmia, the growth of the irradiated animals as compared with non-irradiated controls, was strikingly accelerated. Goldblatt and Soames (50b) found that this same growth effect could be obtained by feeding of rat livers irradiated *in vivo*. The absence of any anti-xerophthalmic activity precluded the synthesis of vitamin A by the radiation, wherefore it was concluded that radiation probably acted as a liberator of stored vitamin. This hypothesis seemed to Steenbock and Nelson (52) to present the paradoxical position that light liberates vitamin A for growth, but not for the prevention of the ophthalmia. They, in turn, suggested that a far more justifi-

able conclusion was that the growth ceased because of an insufficiency of an antirachitic vitamin as distinguished from vitamin A. The experiments which followed in support of this thesis are now well known. Steenbock and his co-workers showed very clearly that radiation was without effect on vitamin A and that cessation of growth on these supposedly vitamin A deficient diets was due to a simultaneous deficiency of the antirachitic vitamin, which, when formed by radiation, stimulated growth. They further showed (53) that Goldblatt and Soames' results with irradiated liver were due to the activation of vitamin D in the liver by the radiation, and suggested that "in ultimate analysis, both light and the antirachitic vitamin may represent the same antirachitic agent . . ." This was the beginning of the modern concept of the relationship between vitamin D and radiation.

There have been several investigations tending to show that rats (54, 55) or mice (56) grow better when exposed to red or infrared radiation, and that there is a certain amount of antagonism between this and the ultraviolet part of the spectrum with respect to growth. Recent experiments (57), however, fail to substantiate these claims that red or infrared radiation exerts any specific effect on the growth of rats.

Harnes (58) found that rabbits kept in darkness and irradiated daily with the quartz mercury vapor lamp showed a better initial growth than the non-irradiated controls, but at the end of 6½ months there was little difference in the two groups, in fact, the weights of the irradiated group tended to be less. Brown (59) carried out a most extensive and interesting series of experiments in which the influence of light-environment on the growth and nutrition of normal rabbits was studied by comparing the weight curves of animals living under different environmental conditions for periods of 4 to 8 months and the effects of change from one environment to another. Prolonged exposure to neon light was compared with confinement in the dark and exposure to diffuse filtered sunlight of varying intensity. The radiation from the neon lamps was chiefly in the red and near infrared (580 to 760 $m\mu$) with bands between 337 and 362 $m\mu$. Albino rabbits kept in this light-environment showed a marked gain in weight over that of the control group. This gain in weight was accompanied by increased proliferative activity of hair follicles over shaved areas of the skin, and by increased functional activity of certain organs. The weight curves of animals exposed to neon light or living in darkness were distinctly different from those of the control animals. Animals placed under either of these conditions showed an immediate and decided increase in weight, the initial rate of

which was much the same in the two cases. In the case of animals in light the gain in weight was continuous, and the results suggested that there was a definite promotion of growth with heightened metabolic activity. Following the early gain of the first month or six weeks, the animals in darkness began to lose weight and the effects suggested a depression or inhibition of metabolic activity. Control animals in diffuse sunlight showed prolonged periods in which one or the other of these effects prevailed. Brown believes that the changes are out of proportion to the differences in the intensity of radiation and regards the constancy or fixity of the light-environment as being the important factor.

There is no question that radiant energy will promote normal growth and development in chickens fed a vitamin D deficient diet, the benefit being proportional to the unfeathered area exposed. Sheard (60), however, believes that visible as well as ultraviolet wavelengths play a part in this action, since chicks exposed to ultraviolet or visible radiation exclusively grow poorly and show abnormal parathyroid development. On the other hand, cod liver oil added to the diet will make up for the deficiency in either part of the spectrum. Much more needs to be known about the metabolism of the chick before these and other results can be correlated with results obtained on other animals.

BASAL METABOLISM

The considerable amount of clinical and experimental work done in this field has failed to consistently indicate any specific effect of radiant energy on basal metabolism. Kestner and his co-workers claim that irradiation leads to an increase in oxygen consumption, but the majority of workers have found very little change or, in some cases, a decrease in oxygen consumption and in basal metabolism (1, chapter 13). As Laurens points out, "A man by a few small muscular contractions per minute can change his metabolism much more markedly than by several hours of irradiation." This is aptly illustrated by a more recent publication by Arnautov and Weller (61). These authors made an intensive study of three subjects exposed for periods of 1 to 3 hours to darkness, daylight and artificial radiation. The maximum increase in pulmonary ventilation and basal metabolism found in changing from darkness to daylight was 7 p. c. Artificial radiation increased the pulmonary ventilation and basal metabolism about 6 p. c. The authors admit that the differences are slight, but believe they are significant in indicating a definite influence of radiation.

Harris (62) claimed that irradiation of rats and mice with the band of radiation of wave-

lengths 291 to 436 m μ resulted in an increase of about 20 p. c. in the carbon dioxide output, but Campbell (63) was unable to confirm these results. Men, mice and rats irradiated with the entire spectrum of the quartz mercury vapor lamp or with only the visible or the ultraviolet showed no changes in basal metabolism.

The attempt to elucidate the part played by radiant energy in the production of the rise in basal metabolism so often observed during insolation of the nude body, has provoked a number of interesting investigations. The consensus of opinion seems to be that of the various climatic factors that favor the cooling of the body and the rise in basal metabolism, such as temperature, humidity, air movement and solar and sky radiation intensity, the rate of air movement is the most important. The rise in basal metabolism produced by irradiation *per se* is insignificant when compared with the change produced by exposure to the cooling effect of the air. Jakawenko (64) divides all of the climatic factors which effect gaseous exchange in man into two groups: (1) those increasing the gaseous exchange, apparently due to the irritation of the peripheral cutaneous nerve endings of the sympathetic, such as, low temperature and air movement, and (2) those factors which decrease the gaseous exchange such as air temperature, absence of air movement and excess of heat produced by solar radiation. The end result of a sun and air bath on the gaseous metabolism would therefore be determined by the specific conditions obtaining at the time of the exposure.

THYROID

It has been suggested at various times that lack of sunlight plays an important part in the causation of human goiter. Bernhard (65) believes that "apparently in the case of endemic goiter there are several etiological factors, and one of these may well be want of light, with all its consequences on the quality of the air, water, soil, flora and fauna." He cites what seems to me to be questionable evidence showing that goiter may be prevalent in a "shady" community and absent in a neighboring "sunny" location, since, in most cases, a change in the water supply was quite effective in alleviating the goiter. In a later section of his monograph he describes the treatment of twelve cases of Graves' disease in which marked improvement was obtained by insolation. We thus have a somewhat paradoxical situation in which the same specific is effective in hypo- and hyperfunction of the thyroid gland.

Smith (66) has restated and amplified Bernhard's conclusions. From a study of data relating to solar radiation and goiter incidence, he

concludes that endemic goiter in the United States, India and New Zealand is the result of a deficiency of solar radiation, which, in turn, tends to produce a deficiency of the iodine content of the gland due to a lack of irradiation of the air, soil, food, drinking water or of the skin of the animal organism; and that the mechanism may be a lack of diffusible iodine, an increase in the goitrogenic factor in certain vegetables, or a disturbed calcium metabolism. This appeals to the writer as an unwarranted oversimplification of a very complex problem.

There is, it is true, an abundance of evidence which indicates that both in humans and in animals there may be seasonal variations in the size or weight of the thyroid gland, the iodine content of the gland, and the iodine content of the blood. One cannot, however, without more definite and positive evidence than is at present available, ascribe these seasonal variations to differences in radiation, and disregard possible effects of temperature, iodine consumption, etc. As Levine and Remington (67) point out, "It seems quite reasonable to expect seasonal changes in the size, histological appearance and iodine content of the thyroid, since the demand for bodily heat production (regulated by thyroxin) is much greater in the cold winter months than in the warm summer months. It might seem to follow, therefore, that the body requires more thyroxin in the winter than in the summer. This, in turn, would create an increased demand for iodine in the diet in order that the gland may manufacture the additional thyroxin. If the diet always contains an amount of iodine greater than the body's requirement, it is more than likely that there will be no seasonal influence of temperature on the thyroid for the reason that the body will always be able to manufacture variable amounts of thyroxin as needed."

Many attempts have been made to experimentally demonstrate a relationship between the thyroid and radiation in lower animals. In the chicken, ultraviolet radiation (68, 69), or ample amounts of vitamin D (69), seem to be essential for the proper development of the thyroid gland. On the other hand, the evidence derived from work on the rat is extremely inconsistent. Bergfeld (70) and others (71, 72, 73) reported the development of a thyroid hypertrophy or hyperplasia in rats kept in darkness or deprived of ultraviolet radiation, particularly of the antirachitic portion. Other investigators, however, have failed to confirm these results (74-77).

In recently completed experiments (48), each lasting from 6 to 16 weeks, I have been unable to find any evidence of a specific relationship between thyroid development and radiant energy. Rats fed a well balanced stock diet and placed,

or born and reared, in darkness showed no difference in the histological picture or size of the thyroid gland when compared with rats kept in diffuse roomlight or exposed to sunlight, carbon or quartz mercury vapor arc radiation. Bergfeld and Bennholt-Thomsen and Wellman, who reported the development of thyroid hyperplasia in rats kept in darkness, fed their animals on bread and milk, a diet which has been shown to be moderately goitrogenic. A second series of experiments was therefore designed to test the possibility that their positive results were due to the fact that darkness enhanced, and radiation prevented or diminished, the hyperplasia resulting from the feeding of a goitrogenic diet. Fifty-five rats, all born in the dark room, were fed a fresh milk and white bread diet for 9 weeks. Various types of exposures were used. Controls were placed in the "light" stock room, other groups were irradiated with the quartz mercury vapor lamp, carbon arc, or with sunlight, using the entire spectrum, the ultraviolet only, or the entire spectrum filtered through window glass. Our results were again negative throughout and failed to yield evidence that darkness enhances the hyperplasia produced by feeding of the goitrogenic (bread and milk) diet, or that radiation prevented its development. On the contrary, the most hyperplastic glands were those in the rats which were exposed to unfiltered sunlight (see also McCarrison (77)).

Bergfeld (69b) reported that when an extract made from the skin of rats irradiated with wave lengths 280 to 318 $m\mu$ was injected into rats kept in darkness, the hyperplasia disappeared and the histological picture returned to normal. Nitschke suggested that Bergfeld's success with the skin extracts was due to the vitamin D formed in the skin upon irradiation with ultraviolet. He accordingly fed irradiated ergosterol (Vigantol) to rats kept in darkness, and reports a disappearance of the hyperplasia and return to the normal histological picture. Bennholt-Thomsen and Wellman found that 0.05 cc. of Vigantol daily for 4 weeks prevented the development of hyperplasia in rats kept in darkness on a bread and milk diet. These results, demonstrating an anti-goitrogenic action of irradiated ergosterol, are difficult to reconcile with those of Thompson (78) and Levine, Remington and von Kolnitz (79) who found that the addition of vitamin D (as viosterol or irradiated yeast) to the Steenbock rachitic (and goitrogenic diet) in no way prevented or lessened the development of hyperplasia. To determine the relationship between vitamin D, thyroid and radiation, a third series of experiments was conducted in which the Steenbock rachitic (and markedly goitrogenic) diet was fed to fifty-five rats for 7 weeks. Supplements of viosterol and

KI were added to the diet of appropriate control groups kept in darkness, others were irradiated with the carbon arc or with the entire spectrum or the ultraviolet part only of the quartz mercury vapor arc. All rats in the dark control groups whose diets were unsupplemented, or supplemented with KI, developed moderate or florid rickets. No rickets was found in the irradiated groups, showing that at least sufficient vitamin D was being furnished to provide for proper calcification. Marked hypertrophy and some degree of hyperplasia were evident in the thyroid glands in all groups except the group fed KI supplement. The addition of viosterol, while protecting the animals against rickets, failed to materially influence the development of the hyperplasia in either direction. Radiation not only failed to prevent, but as in the preceding series, actually enhanced the degree of hyperplasia of the thyroid. If radiation is indeed essential to the proper development of the thyroid gland in the rat, we have thus far been unable to discover the conditions necessary for the demonstration of this fact.

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DISCUSSION

Dr. Harris: I believe that MacLeod obtained some very curious results for the polynuclear counts in people living in various parts of the world, the average polynuclear count of people in one locality being different from the average count for people in another. Is it likely that this is correlated with differences in intensity?

Dr. Ponder: I don't think so, for the highest polynuclear counts were obtained in Florida, where there is plenty of sunlight, but also in the midlands of England, where the radiation intensity is certainly not comparable to that in Florida. I think that the differences must be due to factors other than light intensity, although we don't know what they are. Differences in radiation might, of course, be important if one were considering high altitudes.

Dr. Mayerson: I believe we should be extremely cautious in ascribing geographical differences in function to solar radiation in the absence of definite and accurate information as to its intensity and character at each particular place. I am particularly critical of workers who assume that ultraviolet radiation varies directly as the average total radiation and so ascribe their results to ultraviolet radiation. To one who has measured solar radiation and who knows the extreme variability of ultraviolet radiation with at-

mospheric and sky conditions, such an assumption is absurd. For example, Fresno, California, receives an extremely large amount of total radiation throughout the year, much more than New Orleans, but the antirachitic ultraviolet component is hardly two-thirds as great as in New Orleans. We must also bear in mind that in most places there is very little radiation of wavelengths shorter than 300 m μ even under ideal conditions in summer and not very much that is less than 305 m μ or even 310 m μ during the winter.

Dr. Davenport: I have had some little experience bearing on the effect of radiation in the question of goiters. I studied families in the valleys of Western Maryland with reference to the distribution of goiter, and found families, receiving about the same amount of sunlight, receiving the same amount of iodine in the water, because they drank the same water, yet differing greatly in the incidence of goiter. There were some families where, with twenty in the family, there were no cases of goiter, and others in which a large portion of the members of the family were affected by goiter. There were some cases of intermarriage between members of a family which never had goiter, and members of a family which had goiter. In these, goiter appeared in the first generation of offspring. These facts led to the hypothesis that in seeking the cause of goiter as, indeed, of other effects, the constitution of the individual must be taken into account. We cannot say that absence of sunlight is *the* cause of goiter, or insufficient iodine is *the* cause. In general, most human effects have two or more causes and individual constitution is one of them.

Dr. Ponder: I think that one must be very cautious in interpreting some of the experiments on the red cell response of the marrow and on anti-anæmic substances, if for no other reason than that the white cell response of the marrow is a very complicated one, and because red cells and white cells are derived, after all, from the same progenitors. Some years ago, I and my collaborators irradiated rabbits both with X-rays and with ultraviolet light, and this resulted in a sharp deflection of the polynuclear count, the blood stream being filled with young forms from the marrow within a few hours. But one gets the same thing by burning the skin with a hot iron, or even by only bruising the skin, and the effect does not appear to be specifically associated with radiation. It seems to follow superficial or deep injury of any kind. An even more curious phenomenon occurs under similar circumstances in the cat and the rabbit, and possibly in other animals; the white cell count and the blood pressure steadily fall in an animal in which the skin is cut or injured, and may reach less than 50 percent of their initial value after a few hours. Obviously some

substance must pass from the site of irradiation, or injury, to the marrow, and we came to the conclusion that the substance, or substances, is some protein degradation product. Peptone, nucleic acid, adenylic acid, and other similar substances are capable of acting as powerful marrow stimulants, and as these are almost certainly produced at the site of injury, we may temporarily suppose that they are the marrow stimulants. One very good stimulant for the leucogenic centres in the marrow is the blood of the animal itself, for if some blood is drawn off, allowed to stand, and then re-injected, there is an appreciable deflection of the polynuclear count. If the blood is haemolysed before being re-injected, the marrow response is of enormous magnitude. The situation in human pernicious anaemia may be quite different, of course, from the situation in the normal rabbit. I feel doubtful of the alleged specific effect of stromata nevertheless. One of the most difficult things in the world is to prepare stromata without altering the stroma material to such an extent that reactions obtained with it are physiologically meaningless. We know, of course, that cytolysed white cells provide a very effective stimulus to the leucogenic centres, and it would be very nice if stromata were to provide an effective stimulus to the erythrogenic centres. The point, however, would not be so easily proved as some people think. One has only to look at the history of the so-called "erythropoietins" in order to see what the difficulties are.

Dr. Blum: There has been an unfortunate tendency to assume that photodynamic sensitization is a mechanism similar to that of normal photo-physiological effects produced by ultraviolet radiations shorter than 3200 Å. One may often produce similar results by the two methods, just as Ponder has found with various types of skin injury. We have every reason to believe the mechanism to be entirely different. The idea that they are the same has led to various assumptions. For instance, I recall a case where hematoporphyrin injection followed by irradiation was used in the hope of curing rickets simply because such treatment produced sunburn and pigmentation.

Dr. Climenko: Ponder mentioned an experiment which I should like to elaborate. The exposure of rabbits to ultraviolet irradiation will evoke a leucogenic reaction which is characterized by a marked increase in the number of juvenile polymorphs in the peripheral circulation. The administration of irradiated ergosterol to such animals will produce a similar stimulation. In both instances, the leucogenic response was associated with a marked increase in the serum-calcium level. The administration of irradiated ergosterol to man, however, produces no such effect. A group of patients was studied who were being treated with massive doses of irradiated

ergosterol for such conditions as active rickets, delayed union of fractures, and osteitis fragilitans. None of these patients showed any indication of leucogenic stimulation, nor did any of these patients show a significant change in the serum-calcium level. This suggested the possibility of the original response resulting from the change in the calcium level, rather than from any direct effect of the ultraviolet irradiation. A series of animals did show a definite leucogenic stimulation when either calcium chloride or calcium gluconate was administered intravenously. There was no specificity related to calcium changes, for a similar stimulation could be produced by the administration of iron, copper, cobalt and zinc. In fact, the intravenous administration of small quantities of distilled water was found to be capable of affording an effective stimulus.

Dr. Meyer: It would be valuable to observe the gastric secretion concomitant with the local effects of radiation. If histamine or a histamine-like substance is produced locally, one might expect a stimulation of gastric secretion. This might be correlated with stimulation of the hematopoietic function, in view of the fact that the origin of the anti-anemic factor of liver is in the gastric secretion (Castle's ferment).

Dr. Ponder: Histamine monohydrochloride has no effect on the rabbit bone marrow.

Dr. Bills: Related to the general remarks, I recall the experiments of some of the German and Swiss milk-producing concerns who were prompt to take up the idea of irradiating milk a few years ago. They had trouble at first with irradiated milk showing toxic effects; these were traced to protein decomposition products and not to the vitamin. It was a good example of the protein degradation products that can be produced by exposure to ultraviolet radiation.

A chemist's first thought on the material presented by Mayerson is that radiation, in those instances where it produces distinct physiological effects, duplicates the end results of various potent medicaments. If radiation acts through forming pharmacologically active principles, as it is reasonable to assume it does, our future problem is to learn what they are.

Our present knowledge of one class of active substances which are produced by irradiation came rather suddenly through the studies with vitamin D. Now we know that there are several vitamins D, with differing physiological actions, and in addition that sterol derivatives related to vitamin D have markedly diverse actions as cardiac poisons, sex hormones, bile acids, venoms, and a group of materials which alter the mineral composition of the blood without exhibiting antirachitic action. If all these comprise one closely knit chemical family, how many more active

agents can one expect from the innumerable materials in the body that are capable of absorbing light and being altered by it!

Not to prejudice you with the importance of vitamin D, I may recall a few facts about it which fit with Mayerson's topics. Although basal metabolism is not much affected by direct irradiation, it is undoubtedly increased by massive doses of at least one of the forms of vitamin D. This was shown in the work of Reed with irradiated ergosterol. However, ergosterol is probably not present in the animal body, and therefore that form of vitamin D which an animal receives by irradiation is something chemically different from irradiated ergosterol. It is interesting also to recall Nitschke's experiment which showed that hedgehogs given irradiated ergosterol at the time of their winter sleep did not hibernate, but remained warm and active.

The anti-anemic effect of radiation in tuberculosis reminds me that the very earliest experiment in which an irradiated remedial agent was administered dealt with the anemia of tuberculosis. Thompson in 1854 found that coconut oil, as made in Ceylon (by the sun's rays on copra) was almost as effective as cod liver oil, whereas almond oil and olive oil were without action in the anemia of tuberculosis. Thompson barely missed a great discovery, for which we had to wait three-quarters of a century.

It is significant that iron accompanies calcium in rickets. I wonder that studies on this relationship have been so few. Beard's work of course is well known. Another piece of work not so well known is that by MacCallum, about a decade ago. He found, in examining rats from Dr. Colum's colony, that those with healing rickets showed deposits of iron wherever calcification was seen. The well known line test for rickets can, in fact, be carried out almost as effectively by staining the sections for iron as by staining them for calcium.

Dr. Sheard: Blood pressure and pulse rate are affected by so many factors and, in some instances, so readily, and hypertension is of such varied type or character that considerable care must be exercised in the criteria which are established in experimentation on blood pressure, pulse rate, cardiac output and so forth. However, I believe we may accept as proven the conclusion that, in general, irradiation tends to (1) reduce blood pressure, (2) increase pulse rate and (3) increase cardiac output in man. The possible causes of the reduction in blood pressure have been presented in résumé by Mayerson. From my own experimental observations I feel certain that the inhalation of the combustion products, such as ozone and nitrous oxide, produced by various types of ultraviolet lamps, tends to reduce

blood pressure even though the body is not irradiated. However, judging from the results obtained from various types of investigation which my colleagues and I have carried on, I believe that the chief cause of reduction in blood pressure and other effects produced on the circulatory system is to be ascribed to the dilatation of the capillaries with an increase in the number of open or functioning capillaries of the skin and superficial tissues, thereby producing a decrease in the peripheral resistance to blood flow. Certain measurements that I have made on the differences of potential across specified areas of the extremities, before and after irradiation, indicate quite definitely that the superficial blood flow is enhanced. Without doubt, irradiation of the skin produces a disturbance of the balance between the peripheral sympathetic and splanchnic parasympathetic tone. Again, I believe that the work of Lewis and of Harris quite clearly indicates the liberation of substances with histamine-like properties and action, leading to dilatation and increased permeability of the blood vessels. Hence it is apparent that any such effect as the reduction of blood pressure by radiant energy is not to be attributed to a single factor but rather to several agents or reactions which may vary in different individuals and at different times in the same individual.

Penetrating heat (infrared) radiation from carbon arcs, quartz mercury lamps and so forth would produce, after absorption by the tissues, an erythema which would affect the blood vessels of the superficial and deeper layers of tissue. In general, the effects due to heat (infrared) erythema are more or less transient in character and I am of the opinion that the ultraviolet and near-ultraviolet portions of radiant energy are chiefly responsible for these reactions. At any rate, subsequent to irradiation with sources rich in ultraviolet light I have found that the temperatures of irradiated areas in an arm or leg of a human subject are a degree or two (Fahrenheit) higher than in corresponding areas in the non-irradiated extremity. These thermal differences persist for some days following an erythema dose. Increased temperature is indicative of increased blood supply and, therefore, of increased blood flow or decreased circulation time.

HEMOGLOBIN AND ANEMIA

In discussions relative to the effects of radiant energy on the content of hemoglobin, red cell count and in the treatment of anemia, it should be emphasized that radiation cannot serve as a substitute for dietary deficiencies in the formation of hemoglobin. The same statement is applicable to researches on rickets and calcium metabolism, since it has been shown that radiation is effective

only by virtue of the fact that it serves as an energizing or vitalizing element, thereby enabling the organism to utilize materials which may be present, but in an unusable or unavailable state. Hemorrhagic and hemolytic anemias are markedly different in character. We should expect but little influence of radiant energy in hemorrhagic anemia, since in severe cases the reserves of iron may be depleted whereas, in hemolytic anemia, there is the possibility of the utilization of the iron which is made available by the destruction of the hemoglobin or, again, radiation may serve as an agent to minimize the destruction of hemoglobin. In general, the normal processes and stimuli to hematopoiesis may be present, but it is as impossible for the body, with or without radiation, to make hemoglobin and to counteract anemia without the essential materials as it was for the Israelites to make brick without straw. Therefore, in many of these problems there are three factors of essential importance: (1) adequate supply of materials, (2) materials in an available form, (3) chemical reactions and end products resultant on the absorption of radiant energy.

THYROID AND PARATHYROID GLANDS

In the case of the parathyroid glands of chickens, my colleagues and I (cited by Dr. Mayerson) have shown that normal growth of chickens and normal development, growth and function of the parathyroid glands do not occur under selective regions of the solar spectrum. When young chicks are fed on diets adequate in all particulars except vitamin D, normal growth and normal parathyroid glands are found only when the chicks are fed cod-liver oil or viosterol, or are housed under quartz glass which adequately transmits the visible and ultraviolet portions of sunlight. Chicks housed under amber or blue glass filters developed hypertrophy and hyperplasia of the parathyroid glands; the same results occurred when groups of chicks were housed behind purple Corex glass which transmits little solar energy except in the near ultraviolet region. Adequate exposure of the chicks, housed under amber or blue glass filters, to radiation from air-cooled quartz mercury lamps induced normal growth of chicks and normal growth and development of parathyroid glands (Sheard, Higgins and Foster, 1930).

In 1934 Higgins, Wilder and I reported the results of experiments on the effects of ultraviolet irradiation of rachitic chickens. The effects of diets deficient in vitamin D are shown not only in the bones, but also in the thyroid and parathyroid glands and in the blood constituents (Marine, Nonidez and Goodale, Higgins and Sheard, Turner and Benedict). Our recent investigations

show that ultraviolet irradiation of rachitic chicks produced these effects: (1) The long bones became firm and hard, the cysts of the cortex disappeared and normal calcification was restored. (2) The calcium content of the blood was restored to normal and the calcium-phosphorus ratio was raised from 1.11/1 to 1.9/1, the phosphorus content, however, remaining high. (3) The greatly enlarged parathyroid glands were reduced almost to normal. (4) The hyperplastic thyroid glands resumed the appearance of the normal glands; the follicles were as large as, or larger than, those of the control chickens and were well filled with colloid, and the parafollicular cells were normal in their distribution. These and similar experiments show the dependence of the normal development and function of the thyroid and parathyroid glands on some form of vitamin D.

There is also the interplay between parathyroid hormone and vitamin D in preventing hypertrophy and hyperplasia of the parathyroid glands. Apparently the ability of the parathyroid glands to increase the supply of their products represents a compensatory mechanism which protects the organism against relatively low degrees of deficiency of vitamin D. The parenteral administration of parathormone in minor degrees of deprivation of sunshine or vitamin D (insufficient to cause rickets, but permitting hyperplasia of glands) prevents hypertrophy and hyperplasia. Other observations of Wilder, Higgins and me have led to the conclusion that the supply of parathyroid hormone determines the sensitivity of the organism to the action of vitamin D. By virtue of the capacity of the parathyroid glands to accelerate the rate of supply of their product, and owing to the resulting conditioning of the tissue (increased sensitivity to vitamin D), the organism is enabled to withstand periods of relative deficiency of vitamin D which otherwise would produce rickets or osteomalacia. This compensatory mechanism is adequate to protect against relative degrees of deficiency of vitamin D; it is inadequate, as would be expected, when deficiency of vitamin D is extreme.

In a survey of 134 proved cases of hyperparathyroidism, prepared by Wilder, (in press) it is demonstrated that very few cases have been found in the central (Mississippi Valley) states as compared with England or New England. On the other hand, hyperthyroidism and goiter have been commonly found in the inland regions of the United States, and but infrequently in New England or England. The deficiency of iodine in the diet and the prevalence of thyroid dysfunction and hyperthyroidism in people living in regions in which there is a relatively large number of clear, sunshiny days, points to the conclusion

that solar energy as such is a minor factor in these conditions. On the other hand, hyperparathyroidism may be dependent chiefly on a deficiency of solar energy (particularly in people living in cities and in regions of frequent cloudy days) rather than on calcium and phosphorus deficiencies. By inference from the experiments on animals the suggestion may be ventured that hyperparathyroidism is due to the attempt of the parathyroid glands to increase the supply of their product to serve as a compensatory mechanism and thus protect the organism against relatively low degrees of deficiency of vitamin D. However, the parathyroid glands of the majority of men and women are apparently able to increase functional activity without hypertrophy. Hamilton and Schwartz have given us clinical evidence of increased parathyroid function in children deficiently supplied with vitamin D. There is, therefore, an intimate cycle of products of parathyroid glands, radiant energy or vitamin D, and available supply of mineral ingredients.

Dr. Mayerson: I am not sure that I am willing, at the present time, to go so far as Sheard does in postulating a very close reciprocal relationship between vitamin D and the parathyroid. Although vitamin D seemingly can be used to compensate for lack of parathyroid hormone when the latter is deficient in amount, there is considerable evidence tending to show that the reverse substitution cannot be made.

Dr. Bills: In some cases the vitamin and the hormone may even act antagonistically. It is remarkable how different substances affect calcium metabolism in different ways. Moderate doses of vitamin D cause the deposition of calcium in the bones if the bones need it. Parathyroid hormone elevates the blood calcium particularly, and may even do so at the expense of the bones if the supply in the diet is short. Massive doses of vitamin D, and smaller doses of certain decomposition products of vitamin D, such as toxisterol, act somewhat like the parathyroid hormone. This picture is over-simplified, no doubt, but covers the situation in the fewest possible words.

Dr. Mayerson: In closing this discussion I should like to re-emphasize the work of Brown. His experiments are unique in that the rabbits were exposed continuously to the light environment. The fixity or constancy of the light environment is assumed to be an important factor. This assumption is supported by the fact that we (Mayerson, Gunther and Laurens, *Am. J. Physiol.*, 75, 399; 421, 1926.) found that calcium and phosphorus in dogs were affected by a change from roomlight to darkness, or *vice versa*, in the same way that they were by irradiation with the flaming carbon arc. Crofts and Laurens (*Am. J. Physiol.*, 60, 300, 1924) also found that, in the

frog, absence of light disturbed the respiration in the same way as a short exposure to radiation did. This would seem to indicate that a positive or negative variation in the amount of radiation produces similar changes in function. Additional work (using Brown's method of subjecting the experimental animals continuously to the radiation) under investigation should yield valuable results.

Dr. Hausmann: The presentation of Mayerson is a welcome addition to the excellent volume of Laurens, "The Physiological Effects of Radiant Energy". Somewhat disappointing but pertinent is the statement that up to the present the outstanding successes of light therapy are to be found primarily in the treatment of rickets and of extrapulmonary tuberculosis. At this point—it has also been considered in a footnote by Mayerson—I would again like to recommend that the use of the general expression "radiation" be replaced (when strictly discussing the effects of light) by the designation "light"—or at least "ultraviolet rays". Out of the complete picture presented I may only touch on a point or two, e.g.—our knowledge of "darkness". Our knowledge of this field has been developed, indeed, by Laurens and Mayerson themselves. In agreement with the great physiologist, Jacques Loeb, who carried out his most significant experiments in the United States, I have also pointed out repeatedly that light does not represent a necessity for life in the sense of "Sein" or "Nicht sein" for animal life and humans. Further, as Günzburg of Antwerp has emphasized, we are dealing, in many instances, with organisms which ingest plants grown in light or their products. The extraordinarily interesting recent researches which demonstrated animal life 900 meters deep in the ocean have added new proof that light can be dispensed with in the above sense. Mayerson's presentation of the significance of the ultraviolet rays on flowing blood has been of especial interest to me since it seems to bring us closer to a solution of the old question regarding the direct and indirect action of light. All in all, in contrast to the sharply defined facts derived from the study of light pathology and light therapy, the study of purely physiological effects is still, as ever, very difficult to grasp and to reduce to simple fundamentals. This, I believe, is in agreement with the conception that light is not a "conditio sine qua non" as oxygen is. The most important representations of Mayerson support the validity of this point of view, for they indicate the difficulties of the problems advanced by Mayerson and Laurens themselves.

Dr. Ellinger: I should like to supplement Mayerson's interesting statements by saying that the liberation (Lewis) as well as the photochem-

ical production of histamin and histamin-like substances (H-substances) (Ellinger) which are used to explain the dilation of skin capillaries designated as light erythema, in my opinion, warrant advancing the "histamin hypothesis of radiant energy action". The action of these H-substances on the skin capillaries not only accounts for light erythema but for the decrease in blood pressure which follows irradiation as a result of the diminished peripheral resistance. The recent finding of Rühl, that histamin dilates the coronary arteries, strengthens the explanation of the successful treatment of angina pectoris as being due to the presence of these H-substances in the blood stream. The increase in the minute volume which was observed by Lindhard is also explained by this hypothesis, since, according to the results of Orzechowski, histamin acts on the heart even in a concentration of 10^{-8} to 10^{-9} . Now the question arises whether one is justified in regarding the circulation of such H-substances produced in the skin as responsible for the different effects of radiation on the circulation and on metabolism. This view seems to me to be confirmed by the following observations:

(1) Feldman and Azuma, in pharmacological experiments, have shown that blood of irradiated animals obtained by heart puncture loses its vasoconstrictor power, but that this effect is not present when the skin is covered with lampblack previous to the irradiation. This definitely indicates that the vasodilator substance is produced in the skin.

(2) The capillary dilation can be obtained at places other than those directly irradiated (Cramer and Lewis).

(3) A particularly important argument for the circulation of H-substances in the blood is the increase in the secretion of gastric juice which has been shown to occur after irradiation with artificial ultraviolet (Diehl) and after sun baths (Barone). This result is particularly important, since like the decrease in blood pressure, it is an essential criterion for histamin.

Of the numerous facts which were discussed in Mayerson's paper, the information that leucopenia is a result of irradiation with ultraviolet, is surprising. This observation is in contradiction to observations of numerous other investigators. Also, this observation is not consistent with the "histamin hypothesis of radiant energy action".

Without taking up further details of Mayerson's discussion with respect to the blood and the circulation, I should, nevertheless, like to add an observation by Garot, who found that the decrease of blood pressure which is observed in adults as a result of irradiation does not appear in children. This is interesting because I have found that in children aged 6 to 12 years the sensitivity of the skin to light is about 50% lower

than in adults, a result of particular importance which explains the observation by Garot on the basis of the "histamin hypothesis of the radiant energy action".

To the observations concerning the influence of radiation on the secretion of gastric juice, I should like to add a few remarks about the problem of the effects of ultraviolet radiation on gaseous exchange. This, as is well known, is the sum of the essentially constant basal exchange and the variable increase due to activity. Since the latter is dependent to a considerable degree on outside influences, such as temperature, the experiments on the influence of sunlight are difficult to explain and have led to the contradictory results to which Mayerson refers. Clinicians have inferred that there is an increase in gaseous exchange after irradiation as a result of the observation that there is an increase in appetite after irradiation with sunlight or artificial ultraviolet, although conclusive experimental evidence has not been obtained to support this assumption. The clinical observation of increased appetite after irradiation has been experimentally confirmed, as indicated above, by Diehl, who found an increased gastric secretion after irradiation. The increase in gaseous exchange suggested by these experiments has been demonstrated by Lehmann and by Lehmann and Szakall. These experiments were carried out with a careful elimination of all factors apt to confuse the picture of the radiation effect, in particular by keeping a constant room temperature and by using an artificial source (mercury vapor lamp). Healthy people were used for the experiments. Along with the production of an erythema by short intensive radiation there occurred an increase in basal metabolism, while chronic irradiation led to a 10 to 15 p. c. decrease.

While, on the basis of the "histamin hypothesis of radiant energy action", one may interpret the increase in basal metabolism as due to an increased activity of the thyroid gland because of better circulation of blood through it and because of the circulation of H-substances in the blood, nevertheless the nature of the chronic action is still very obscure. These experiments further showed that the respiratory quotient, for a mixed diet, increases from a value of 0.75-0.85 to over 1, signifying increased oxidation of carbohydrates. The observation that ultraviolet radiation prevents the decrease of the respiratory quotient resulting from bodily work, which according to Simonson represents the expression of glycogen poverty of the body as a result of work, is quite consistent with Pincussen's recent demonstration that the well-known decrease in blood sugar after ultraviolet radiation is connected with a glycogen fixation in the internal organs. Evidently the glycogen fixation by the irradiation is able to counteract the glycogen decrease due to the mus-

cular activity. This makes it easier to understand the results of Lehmann and Szakall who found an increased ability (up to 60 p. c.) to do work on the ergometer after ultraviolet irradiation. I have discussed these results rather in detail because of their practical importance. They are observations which in this age of sport should interest the layman very much.

As to the statements of Mayerson on the importance of irradiation for animal growth, I can only partly agree with his conclusion that the results given fail to show any influence. Undoubtedly mammals can withstand light deprivation for a long time if they have sufficient (vitamin-containing) food. In connection with experiments by Ludwig and V. Ries on the influence of visible radiation on growth, the observation that among the animals brought up in absolute darkness there were some, which at the end of the observation period, showed the largest weights, seems to me, however, important. They found that mice irradiated exclusively with red light first showed an increased growth in length, while later, undoubtedly due to lack of vitamin D, they died. Evidently the initial increase in growth in these experiments is a result of the lack of irradiation. If one now compares these observations with the facts given by Mayerson one arrives at the conclusion that lack of light, together with sufficient food, increases growth to a certain extent. In this connection I have observed in travelling in Scandinavia a great frequency of very tall people and in general the very large people are found in regions where they have a long winter, as compared with the much smaller people in the Southern part of Europe, such as Italy. It seems to me that something similar is also seen among members of the Mongolian race, because according to the few observations which I have had occasion to make, the Chinese in the northern part of the country seem in general to be heavier built than those in the southern part. Although I am quite aware that on the basis of these observations only hypothetical conclusions can be derived which can be proved only by measurements in a large number of cases and by a

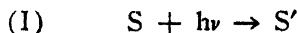
statistical treatment of the data, etc., nevertheless it seems to me that here perhaps is a suitable way to understand the still open question as to the influence of irradiation on animal and human growth. The question as to the importance of the influence of irradiation on animal growth emphasizes the great difficulty in the explanation of animal experiments in general and especially in the field of experimental radiation therapeutics, to which I have referred elsewhere. (Strahlentherapie, 47, 12, 1933). The judgment as to the effect of ultraviolet radiation on the thyroid gland seems equally difficult. As one may conclude from the interesting and voluminous experiments which Mayerson presents, radiation cannot prevent hypertrophy of the thyroid. Nevertheless, one can readily show that there is a definite effect of irradiation on the thyroid glands. Other workers, as well as myself, have observed that a strong solar or mercury vapor lamp exposure precipitates a motor disturbance, which may develop into a definite state of excitation and undoubtedly is an acute thyreotoxic symptom. These results can easily be explained by means of the histamin hypothesis, since, as I have already explained, histamin leads to an important increase in the circulation of blood and therefore to an increase in function of the thyroid gland which is evidenced in the same manner as in a gland known to be definitely hyperfunctional. If one considers the contradictory experimental results reviewed by Mayerson, it is not possible to believe that lack of light and especially lack of ultraviolet is an etiological factor in the production of endemic goiter as Bernhard believes, and I can agree with Mayerson in summarizing the goiter problem when he says: "If radiation is indeed essential to the proper development of the thyroid gland of the rat, we have thus far been unable to discover the conditions necessary for the demonstration of this fact." This understanding can stimulate biologists working in the radiation field to try to explain this relation between radiation and the development of goiter, since it is particularly true in the field of experimental radiation therapy that "Here are difficulties to be overcome".

PHOTOSENSITIZATION OF LIVING SYSTEMS

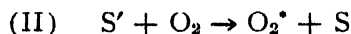
HAROLD F. BLUM

The type of photosensitization described by Oscar Raab (22) in 1900 has been most often referred to as *photodynamic action*, a term which implies a more general importance than can be attributed to the phenomenon. The term is convenient, however, and I shall employ it in my discussion to designate this particular type of photosensitization. Essentially, the phenomenon is the production of changes in biological systems by light acting upon a photosensitizing substance which has been added to the system. The photosensitizers include a wide range of natural and synthetic dyestuffs; the changes produced are usually destructive. While the attempt has been made to explain many types of photobiological phenomena in terms of such reactions, these attempts have generally failed. It will be the purpose of this paper to consider the mechanism of *photodynamic action*.

Since the radiation which produces such phenomena is limited to those wave lengths absorbed by the particular sensitizer (see 6 and 7), the first act in the process must be the activation of the sensitizer molecule S.

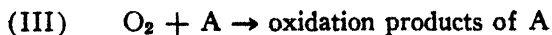


The reactions occur only in the presence of O_2 (see Blum and Spealman, 8 and 10), which must thus be a component of some reaction following (I). We may write schematically:

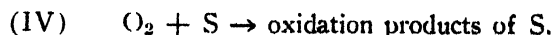


where we wish to indicate that the O_2 molecule is induced to participate in certain reactions, but *not* to indicate a direct transference of the activation from S' to O_2 .

The possible steps involved in (II) are numerous. For example, S' might combine with or transfer its energy of activation to (a) O_2 , (b) an oxidizable substance which might then react with O_2 , (c) H_2O , since these reactions always occur in aqueous solution. Other possibilities might be enumerated, but there is little basis for a choice at present. The first possibility, combination of S' and O_2 , would seem improbable from the observations of Gaffron (13) and Blum and Spealman (8). We must await further studies of the reaction of these dyes in aqueous solution before we can decide upon the mechanism of these secondary reactions. We do know that in simple aqueous solutions containing the dye S, and an oxidizable substrate A, oxidation of the substrate may occur:



and that coincidentally the dye is oxidized, i.e., irreversibly bleached.



It has not been generally recognized that this latter reaction (IV) occurs to an appreciable extent, since it is very difficult to detect the disappearance of the dye by simple inspection. It is readily revealed by spectrophotometric examination, however, (see 8). This bleaching of the dye is obviously an important factor to be considered in studying the kinetics of such photo-oxidations, and has, moreover, a particular importance in photodynamic action, as will be pointed out below.

Although it would seem a relatively simple matter to study the kinetics of such photo-oxidations, there are certain difficulties which must apply to the study of either model reactions, or of photosensitized living systems. If a spectral continuum is used the absorption spectrum of the dye becomes of great importance, and any change in this must modify the rate of the photochemical process. The concentration of the dyes, and hence their absorption, is, of course, constantly changing due to reaction (IV); and, moreover, shifts in the absorption spectrum may occur in the process of bleaching of some dyes, e.g., fluorescein (Wood, 28). Furthermore, many of the dyes are indicators, and changes in hydrogen ion concentration accompany the bleaching process in at least certain cases. Such difficulties could be eliminated, in part, by the use of monochromatic radiation, but unfortunately these reactions occur very slowly except at high intensities, which it is difficult to supply with monochromatic sources. Thus, few or none of the existing studies have been made with monochromatic light.

More important than the above factors, however, is the fact that there is a falling off in the rate of oxidation when the dye concentration is increased above a certain optimum. This may depend on two factors; (a) most of the radiation may be absorbed in the first layers of solution where the O_2 concentration may be so low that all the activated molecules cannot have the opportunity to react chemically before they are deactivated by fluorescence or, (b) deactivation of activated molecules may occur by collision with other dye molecules. Gaffron (14) and Bowen and Steadman (11) find that such deactivation does not occur in the case of oxidation of Rubrene in acetone where the quantum yield approaches unity as the concentration of Rubrene increases. The reactions are probably not comparable; Rubrene forms a peroxide by direct combination with O_2 , which apparently does not occur in the case of the photodynamic dyes. In the case of the photodynamic dyes in aqueous

solution, the quantum yield must fall off as the concentration increases beyond a certain value.

The matter is further complicated by the fact that the rate of the photo-oxidation also enjoys an optimum at a given O_2 concentration. Thus, as shown by Weigert (25) and by Blum and Spealman (8), the rate of oxidation is decreased at low O_2 partial pressures, and again at high O_2 partial pressures. This effect may be explained by assuming that O_2 affects the rate of two different processes: (a) the O_2 molecules inhibit the primary photochemical process, probably by deactivating activated sensitizer molecules without resultant chemical reaction—at high concentrations of O_2 this effect would predominate; (b) O_2 being a component of some secondary reaction following the activation of the sensitizer molecule, the rate of this reaction increases with increase of O_2 concentration—at low concentrations of O_2 this effect should predominate. Such a scheme would not be easily reconciled with any hypothesis of transference of activation from S' to O_2 or direct combination of these substances.

The optimum O_2 concentration probably varies with the concentration of dye molecules and thus there should be an optimum O_2 concentration for each dye concentration, conditions being still further affected by the kind and concentration of the oxidizable substrate and other molecules. Thus in living systems the rate of photo-oxidation of a given component of the system should be a rather complex function.

Although we cannot hope to follow the course of the photochemical reactions in photosensitized living systems, we may make certain generalizations from the facts represented in our scheme. Presumably oxidation of cellular components may occur analogous to reaction (III) which may result in damage to the cell. This must certainly be one of the factors operative in producing photodynamic phenomena. Up to the present, no really quantitative studies of the rate of oxidation in living systems have been made, with the exception of those made recently by Wohlgemuth and Szörenyi (26, 27). The results of these investigations indicate that it may be possible to measure the O_2 uptake in such systems and to separate that part due to tissue respiration from that due to photo-oxidation. The latter appears from their experiments to have a very low temperature coefficient, as is to be expected from its photochemical nature, to be accelerated by HCN whereas normal tissue respiration is inhibited, and to have a ratio of CO_2 production to O_2 consumption far below those for the materials oxidized in normal metabolism. This latter fact gives no help in determining what cell components are oxidized in the photodynamic process, but suggests that the oxidations which occur are incomplete.

Before discussing further the nature of the

photo-oxidations which occur in living systems we may consider another important factor in photodynamic processes which has had very little recognition. This is the activity of the photodynamic sensitizers as a whole, in producing destructive changes in living systems even in the absence of light. These changes are very similar to the changes produced by the sensitizer and light, except that they only occur with relatively high concentrations of the dye. Jodlbauer and Haffner (15) called attention to the fact that those dyes which are most active in producing hemolysis when not irradiated are likewise most active when irradiated. That is, if we compare two dyes, the one which produces hemolysis when in least concentration in the absence of light, will also produce hemolysis in least concentration when irradiated. Considering the complexity of the factors affecting the rate of oxidative processes brought about by these dyes, as we have described them above, this relationship is striking. Indeed, there seems no proper basis for making a comparison, and yet the relationship seems to hold in a general way at least.

One might suspect from this that the dark reaction (Dunkelwirkung), as it has been called, represents an oxidation which is simply accelerated by the activation of dye molecules by light. However, Blum and McBride (5) were able to show in the case of eosine, that the dark reaction proceeds in the absence of O_2 which is necessary for the photoreaction. Furthermore, Wohlgemuth and Szörenyi (27) found that rose bengal has no effect upon the O_2 consumption of red blood corpuscles in the dark, whereas in light it is greatly increased. Hematoporphyrin does increase the O_2 consumption of intact red blood cells in the dark, but not of hemolyzed cells, indicating the relationship of this O_2 consumption to normal cell respiration; whereas the increase of O_2 consumption in the presence of light is the same whether the cells are hemolyzed or not. This evidence would appear to demonstrate that the dark reaction and photoreaction are quite separate processes.

To investigate the correlation between dark reaction and photoreaction we have studied a model system consisting of red blood cells, fluorescein dyes and H_2O_2 , following our earlier investigations (Blum, 4). We have determined the concentration of dye and H_2O_2 in mixtures which will just produce hemolysis after six hours in the absence of light; fig. 1 presents our results. We note first that without H_2O_2 fluorescein exhibits no dark reaction in concentrations up to 1×10^{-2} M, which is about the upper limit of its solubility. However, eosine produces hemolysis at 5×10^{-2} , erythrosine at 5×10^{-4} , and rose bengal at 5×10^{-5} . With increasing H_2O_2 concentration the minimum concentration of dye

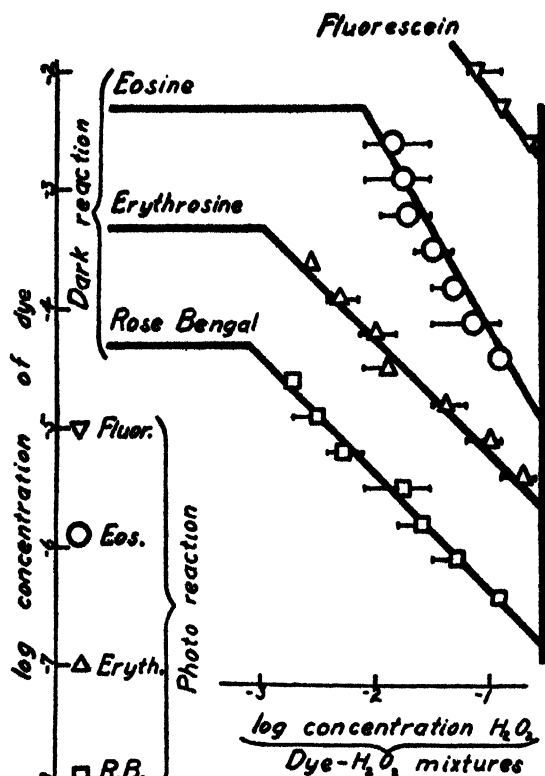


Fig. 1. Dark reaction—represents concentrations of fluorescein dyes which produce minimum hemolysis in the absence of light; fluorescein does not produce hemolysis in any concentration. Dye- H_2O_2 mixtures—points are average values; the horizontal lines indicate the spread of individual determinations. 0.5 H_2O_2 produces hemolysis without the addition of dye. Photo reaction—minimum dye concentrations producing hemolysis after one hour irradiation with sunlight, average values. All mixtures made up in sodium phosphate mixtures pH 7.6 — 7.7, ionic concentration 0.3 M.

which produces hemolysis is reduced until at a H_2O_2 concentration of 2.5×10^{-1} M hemolysis occurs with no dye. The logarithms of the concentration were plotted in fig. 1 in order to condense the data. Allowing for a considerable experimental variation, it may be seen that the curves relating dye and H_2O_2 concentrations are approximately straight lines on this logarithmic scale and the relationship may be roughly expressed:

$$(V) \quad [S]^a \times [H_2O_2]^b = K$$

The accuracy of our data would not permit an attempt to explain this relationship but it would appear to indicate more than a simple summation of the dark reaction of the dye with the oxidative action of the H_2O_2 .

In fig. 1 average values are given for the minimum concentrations of dye at which hemolysis

occurs following exposure of cells and dye to bright sunlight for one hour. It will be seen that the same relationship holds as for the dark reaction—a demonstration of the correlation pointed out by Jodlbauer and Haffner (15). However, if we may draw an analogy between our model and photodynamic hemolysis, we must assume that cell destruction should be measured by the product of the dark reaction and the quantity of photo-oxidation which occurs during the period of irradiation. The latter should, of course, be dependent upon the rate of photo-oxidation which again should be some direct function of the dye concentration, but, as pointed out above, must vary widely with the experimental conditions. We can only point out that the dark reaction of the dye must be considered as a very important factor in the photodynamic process. Wohlgemuth and Szorenyi (27) have shown that the rate of O_2 uptake of red blood cells sensitized with hematoporphyrin is several times as great as for red blood cells sensitized with rose bengal, whereas the latter is in general more active in producing hemolysis and other destructive effects. If, as assumed above, the amount of destruction is represented by the product of the amount of dark reaction and the amount of photo-oxidation, both of which are direct functions of the dye concentration, it is not surprising that we find rather definite lower limits for the concentration of dye producing photodynamic hemolysis (see fig. 1), even though light conditions are somewhat variable, for under these circumstances the magnitude of the destructive action should increase exponentially with the concentration of the dye.

Before leaving the subject of the dark reaction it must be mentioned that fixation of cells may be produced by the photodynamic dyes and that this effect, like hemolysis, is enhanced by either H_2O_2 or exposure to light. Both fixation and hemolysis are markedly affected by hydrogen ion concentration (Blum, 4), by hypertonic salt solution (Bier and Rocha e Silva, 2) and probably by a number of other factors. This would indicate again the importance of the dark reaction in the total photodynamic process as the photo-oxidation would not be expected to show such marked changes with these factors.

Whether a real correlation between our H_2O_2 model system and photodynamic action exists may be worth consideration. Blum and Spealman (8) have demonstrated that H_2O_2 is formed when the fluorescein dyes are irradiated alone in aqueous solution, but we may question whether H_2O_2 is always an intermediate in the oxidation of cell substances. Certainly, the concentrations of H_2O_2 employed in the model could hardly be reached in the photodynamic process, as will be seen by reference to fig. 1. Wohlgemuth and

Szörenyi (27) found that 2 cc. of hemolyzed red blood cells sensitized with 6×10^{-4} rose bengal absorbed only 6 cmm. of O_2 in 30 minutes when illuminated with a 75 watt lamp. This would represent the formation of about 4×10^{-6} M H_2O_2 , a concentration which cannot compare with the concentrations used in the model, but may be a low estimate for the total H_2O_2 formed because any H_2O_2 formed and destroyed by the action of catalase would not be included (see below). However, in the model a great deal of H_2O_2 is destroyed by the action of catalase in the blood cells, and if we could assume that the H_2O_2 acts immediately in the local region where it is produced by photochemical action before catalase has an opportunity to destroy it, we might explain the discrepancy.

The effect of HCN on photodynamic action and on the H_2O_2 model is of interest with regard to this question. Bier and Silva e Rocha (1) have recently published results showing that photodynamic hemolysis is augmented by the presence of 3×10^{-2} and 3×10^{-3} M KCN. Although the results have been somewhat variable, we have found in our laboratory that 5×10^{-4} M NaCN augments both photodynamic hemolysis and hemolysis by eosine and H_2O_2 , but 5×10^{-2} M NaCN inhibits the same effects; at intermediate concentrations NaCN seems to have no marked effect on either process. These differences in the effect of HCN at different concentrations may explain the apparently conflicting findings that have been previously recorded (Loeb, 16; Cooke and Loeb, 12; Moore, 21; and Blum and McBride, 5). We will not attempt to explain the inhibitory effect of high concentrations of HCN at this time, but will be concerned only with the augmenting effect of low concentrations, which is quite striking and seems to have a direct bearing on the question with which we are concerned. Since HCN inhibits catalase which catalyzes the destruction of H_2O_2 , we have a simple explanation of its augmenting action in the case of the H_2O_2 model, for the presence of HCN must be equivalent to an increase in the concentration of H_2O_2 . If we assume H_2O_2 as an intermediate in the photodynamic process we may attribute the augmenting action of HCN in this case to the same factor.

The finding of Wohlgenuth and Szörenyi (26, 27) that HCN (10^{-3} M to 10^{-4} M) greatly increases the uptake of O_2 by photosensitized tissues is an apparent confirmation of the above findings. These investigators used the manometric method of Warburg in measuring O_2 uptake. If H_2O_2 is formed as a result of the photoprocess, a part of it must be constantly broken down by the action of catalase in the tissues resulting in release of O_2 . If the action of catalase is inhibited by HCN this process would be diminished and the apparent uptake of O_2 , as measured

manometrically, would be increased. Wohlgenuth and Szörenyi's findings would be most easily explained on this basis, and indeed it seems difficult to find another explanation.

That HCN does not directly affect photosensitized oxidations is shown by the experiments of Meyer (18, 19) who found that oxidation of pyruvic acid and of various unsaturated organic compounds, by light and chlorophyll or eosine, was not affected by HCN. The photo-oxidation of ergosterol by these sensitizers was inhibited (Meyer, 20) by HCN, but in this case an oxidation occurs in the absence of light which is apparently catalyzed by impurities, and it is probable that the inhibitory action of HCN is exerted at this point. At all events, it appears that the augmenting action of HCN occurs only in living systems, and we must attribute it to the inhibition of some enzyme in these systems, of which catalase would seem to offer the only possibility. Catalase is, of course, a very specific catalyst for the destruction of H_2O_2 , and does not catalyze the breakdown of other peroxides or peroxy-acids (see Stern, 23).

Another important factor to consider is the action of the products of photo-oxidation of the sensitizing dyes (equation IV). Fluorescein dyes irradiated in appropriate aqueous solutions and subsequently introduced into biological systems may produce effects quite comparable to those resulting from irradiation of the dye and biological system together, e.g., cytotoxicity of sea urchin eggs (Moore, 21), hemolysis of red blood cells (Blum, 3; Blum and Spealman, 9; Menke, 17), and production of stasis in frog's mesentery (Teather and Schechtman, 24). The experiments of Blum and Spealman (9) indicate that such effects are not due to the production of oxidizing substances in these solutions, e.g., H_2O_2 .

Menke (17) has recently suggested that such destructive effects are due, in the case of fluorescein, to the production of photocompounds of unknown chemical constitution, which are produced when these dyes are irradiated. The existence of these photocompounds is based upon a certain shifting of the absorption spectrum of this dye accompanied by a damping of fluorescence when the dyes are irradiated in the presence of O_2 , which was originally described by Wood (28). The status of such compounds may be open to question; the apparent shift in the absorption spectrum is due, principally, to the damping of fluorescence. The shift in the absorption spectrum is most evident in those dyes showing the greatest fluorescence. Thus fluorescein shows a considerable shift, whereas this is apparently absent in the case of rose bengal. Furthermore, the photocompounds of other photodynamic dyes have not been demonstrated. For these reasons I shall speak collectively of the oxidation products

of the dyes, including the photocompounds, as possible components of this group.

As we have said above, *photodynamic* effects occur only in the presence of O_2 , and, furthermore, they are prevented by the presence of readily oxidizable substances, both of which facts indicate the oxidative nature of the changes involved. We see, however, that both the production of bleach products of the sensitizing dyes, and direct oxidation of cell constituents are affected by these conditions.

It is difficult to evaluate the importance of these bleach products. We have performed a number of experiments in which the dyes were bleached in sunlight, the concentrations of dye determined spectrophotometrically and the concentration of bleach products estimated by subtracting the concentration of dye left, after irradiation, from its original concentrations. So far as they go, these experiments do not indicate a very great toxicity for the bleach products, nor do they indicate that they are much more toxic for one fluorescein dye than another. However, we have no idea of the nature of these bleach products, and the toxicity of the irradiated solution may depend upon the degree of oxidation which has taken place. Moreover, by assuming that they are more destructive when formed *in situ*, we might account for this discrepancy.

The hypothesis that these oxidation products are the destructive factor in *photodynamic action* fits the experimental evidence discussed above. They are formed only in the presence of O_2 . Their formation should be inhibited by catalase if H_2O_2 is an intermediate in this type of photosensitized oxidation, and hence IICN should augment their formation and thus photodynamic action. Moreover, we might account for the relationship between H_2O_2 and dye concentrations represented in equation (V) by assuming that the H_2O_2 oxidizes the dye stoichiometrically and that minimum hemolysis represents the formation of a given quantity of oxidation products. Against this is the fact that these dyes are not bleached by H_2O_2 at the pH at which the experiments were conducted (pH 6.7). The possibility remains that some cell constituent may catalyze the oxidation of the dye (Blum and Spealman (8) found that this reaction is activated by light).

At the present time it seems advisable to admit both oxidation of cell constituents and the production of toxic bleach products of the sensitizer as factors in the total photodynamic effect, the evaluation of the relative importance of the two being impossible at the present moment. It is quite possible that one of these factors may dominate in some examples of *photodynamic* action and the second in others. In all cases, the dark reaction would appear to be an important factor which must be taken into account.

From a quantitative standpoint the study of photodynamic action would not appear to be far advanced. A knowledge of the various factors involved would seem, however, to give us a foundation upon which to build quantitative experiments.

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DISCUSSION

Dr. Ponder: Since Blum came down here this summer, he and I have obtained a number of results bearing on these photodynamic reactions, and as he has not mentioned them in his paper, I think it will be well if I bring them up in the discussion.

The first point concerns the "dark reaction" between the photodynamic dye and the red cell membrane. We apparently have a state of affairs in which the dye can act as a lysin *per se* when in sufficient concentration, and in which it can act as a lysin in much smaller concentration when the system is exposed to light; putting this in conventional terms, we can say that the dye is a haemolysin which is accelerated by photo-oxidation. In a sense, therefore, the light reaction is only a special case of the "dark reaction," and so it is advisable to begin by studying the kinetics of the latter rather than those of the former.

Selecting rose bengal because of its great hemolytic activity, and using washed rabbit red cells in isotonic NaCl buffered with M/15 phosphate to pH = 7.0, we find that the kinetics of the haemolytic process are identical with those of lysis by simple lysins such as saponin. The time dilution curves are of the usual form and are described by the usual equations with a value n of about 2.0. The lysis is inhibited by serum and, presumably, by liberated cell contents, for the percentage

haemolysis curves show the same sort of skewness as in the case of saponin. The velocity of lysis increases with increasing temperature, and we have been able to establish that the μ -value is about 30,000, just as in the case of saponin lysis; if it were not for the color of the dye, in fact, one might think that one was working with saponin itself. Our study of the kinetics, of course, has been far from exhaustive, but I doubt if anything essentially new would emerge from a more extensive investigation of the "dark reaction," although it might be interesting to explore further into the kinetics of the "light reaction" in view of the relations between dye concentration and H_2O_2 concentration which Blum has described.

The second point is more fundamental. Knowing that rose bengal is a simple lysin, and knowing that all simple lysins convert the discoidal red cells into spheres just before haemolysis takes place, we examined the cells in rose bengal systems in the expectation that the shape of each cell would suddenly change just before it haemolysed. The observations were made in washed rabbit red cells in NaCl-buffer and in an uncovered drop, for a reason which I shall explain directly. We were astonished to find that in such preparations all the cells were perfect spheres, and that this spherical form was obtained in dilutions of rose bengal as great as M/10⁶. Diffraction measurements showed the cell volume to be unchanged, and so here we have a substance which changes discoidal red cells over to spheres without a change in volume. Here I should like to emphasize that M/10⁶ rose bengal is far too dilute to bring about lysis in the diffuse light of the laboratory, although it is a concentration in which lysis occurs in systems irradiated with sunlight.

Such disc-sphere transformations without change in volume are not unknown. 1) They are characteristics of every lysin, and occur just before lysis takes place; the transformation observed with rose bengal, however, occurs in dye concentrations far too small to produce haemolysis. 2) The disc-sphere transformation occurs when small quantities of lecithin are added to the medium bathing the cells; this transformation, however, is unique in that it occurs even if the medium is serum or plasma. 3) The most remarkable disc-sphere transformation takes place if a small drop of red cell suspension is placed between a slide and closely applied coverglass. It is for this reason that the cells in rose bengal systems have to be examined in an uncovered drop. If the drop were covered, the cells would be spherical even in the absence of rose bengal. Although the phenomenon has been studied for years, virtually nothing is known as to what brings it about. It is unlike the rose bengal transformation in that the latter occurs in an un-

covered or hanging drop, but the two phenomena are similar in that both occur in saline only, and that in the case of both the spheres promptly turn back into discs when serum or plasma is added.

If spheres are produced from discs by adding the cells to $M/10^6$ rose bengal, the spheres are again transformed into discs by the addition of serum in such quantity as to produce a serum dilution of about 1 in 500 in the system. The discs are certainly somewhat irregular in shape, but the transformation is nothing short of startling. It is also possible to reconvert the spheres into discs by simply diluting the system with saline, i.e., it seems that the spherical form is dependent on there being sufficient dye available, and that this amount can be reduced either by dilution or by the addition of serum (which combines with the dye), with the result that typical discs appear.

This reversible disc-sphere transformation occurs with fluorescein ($M/10^2$), eosin, ($M/10^3$), and erythrosine ($M/10^4$) as well as with rose bengal, or also with haematoporphyrin ($M/10^5$), but not with methylene blue. The fact that the different dyes of the fluorescein series produce the same effect in different molar concentrations shows that a specific effect is exerted by the dye molecules. It is interesting, nevertheless, to take the most active of the dyes of the series (rose bengal), and calculate the relations between the number of dye molecules present and the extent of the cell surface. If we suppose that the rose bengal molecule is of the same order of magnitude as the sodium oleate molecule, we find that the spherical form is produced in concentrations of dye in which there are barely enough molecules to cover the red cell surfaces; indeed, we have to make rather extravagant assumptions to get anything of the order of a monolayer.

These observations throw, I think, considerable light on the haemolytic action of the photodynamic dyes of the fluorescein series, for they provide us for the first time with evidence that the sensitization of the red cell is accompanied by a gross change in the nature of the surface. I think that most of us have thought of the sensitization as a phenomenon of rather a vague kind; as a matter of fact, it is accompanied by a visible change which probably corresponds to a complete alteration in the molecular configuration of the surface. This change, I ought to again emphasize, takes place in the dark and in this respect the new observations are completely in agreement with Blum's idea that the "dark reaction" is the fundamental one.

I need scarcely point out that this shape transformation is of the greatest significance in connection with the structure of the red cell mem-

brane, for, taken together with what is already known about shape transformations, the observations lead to three conclusions. 1) Since the reversible disc-sphere transformation involves a change in surface area of about $L \pm 25$ p.c., the surface membrane must be a liquid film. 2) The change of shape must be due to effects which operate at the surface of the cell, and not in its interior, for the changes occur when there are barely sufficient molecules of dye to cover the surface. In this connection it may be remembered that lecithin and the photodynamic dyes which produce the disc-sphere transformation have molecular weights of such a magnitude that their penetration into the cell is unlikely, and, further, that the re-conversion of sphere into disc is brought about by serum proteins, the entry of which into the cell is a remote contingency. 3) After the change of shape, the permeability properties of the red cell membrane are scarcely altered, and the volume remains the same. Putting it another way, the area of the membrane, with a consequent rearrangement of its molecules, can change by as much as ± 25 p.c. without its permeability properties undergoing any great change, which is, however one looks at it, a remarkable conclusion.

Finally, and in the same connection, it should be pointed out that the electrical properties of the red cell membrane, in so far as they have been studied, seem to be independent of the change of form, for the capacitive component of the impedance of the surface, per unit area, and also its frequency dependence, is substantially unaltered when the discs are transformed into spheres by the addition of lecithin. If we admit, as is generally done, that the thickness of the membrane can be calculated from the capacity data, this means that the thickness of the surface layer is unaltered when the discs are transformed into spheres, although the reduction of area is about 25 p.c. It will be interesting to have similar observations on the capacity per unit area when rose bengal is used to effect the transformation from disc to sphere, and when serum is added subsequently to turn the spheres back again into discs. These observations will no doubt be amplified by determinations of electrophoretic velocity, for the evidence at the moment is that the transformation is not accompanied by any detectable change in ζ -potential.

Dr. Meyer: In Ponder's experiments the effects of serum on the sensitized red cells is probably explained by the adsorption of the dye by the protein molecules. The physiologically active molecule may be the leuco-dye, both in irradiation and in the dark. This assumes that the same reaction, dehydrogenation of a substrate and reduction of the dye, takes place in the dark at a slow

rate. The effect may be caused by the greater permeability of the leuco-dye into the cell membrane because it is a stronger acid. One should study the effect of leuco-eosine on cells.

Dr. Blum: The leuco-dyes of the fluorescein series are not formed in appreciable concentrations during photo-oxidation in the presence of O_2 . They are formed when the dyes are irradiated together with suitable substrates in the absence of O_2 . The photodynamic effects, on the other hand, occur only in the presence of O_2 so that the leuco-dyes could not be producing these effects. During irradiations of a photodynamic system in the absence of O_2 , leuco-dye should be formed, and thus if Meyer's suggestion is correct, we would expect to find greater destructive effects, e.g. hemolysis, than in the same system in the dark. This is not the case, as has been demonstrated by numerous investigators. Hence the dark reaction cannot be reasonably accounted for as due to the leuco-dye.

Prof. Wohlgemuth: The work of Blum has interested me, particularly the explanation he gives for my discovery of the effect of cyanide on photo-oxidation. I must admit that, up to now, I have not been able to explain, myself, the fact that in my experiments HCN increases photo-oxidation in so conspicuous a way. The explanation which Blum gives is that in animal tissues catalase always tends to destroy H_2O_2 as it is formed by photo-oxidation. Thus the amount of oxygen consumed seems to be smaller than it is in reality. However, in the presence of cyanides, the catalase is excluded. All that seems to me to be a perfectly likely explanation for the HCN effect. Perhaps one would be able to demonstrate this fact in a model experiment without using animal tissue, by using methylglyoxal, pyruvic acid, or another organic substance in the presence of a photosensitizer in the usual way; and in parallel experiments add catalase. Catalase is comparatively easy to prepare in pure form, according to the description of Zeile and Hellström (*Z. physiol. Chem.*, 192, 171, 1930; 195, 39, 1931). Then, of course, at the correct pH concentration, the amount of consumed oxygen will be smaller than in the control. By adding HCN to the catalase experiment, the difference should again disappear. In this way the beautiful explanation by Blum would be definitely proven.

Dr. Meyer: In my experiments on the oxidation of pyruvic acid with haemin and eosine (supplementing the published results mentioned above), the photosensitized oxidation seems to be increased in the presence of HCN, especially if one adds the amount of O_2 consumption in the dark, which is suppressed by cyanide. The increase in oxidation was still more marked with

phenol. Both HCN and phenol are not oxidized by themselves in the systems investigated. I considered the increase in oxidation in the presence of HCN as a case of an induced reaction.

Dr. Blum: Was the oxygen uptake greatly increased? Wohlgemuth and Szörenzi found an effect of considerable magnitude.

Dr. Meyer: The increase is not great.

Dr. Singer: Blum's paper and the discussion by Ponder bring up the subject of the morphological changes that the photodynamic action causes in cells. For a number of years I have been interested in the morphological expression in the living tissue caused by the photodynamic action of substances having strong fluorescence in the visible spectrum after illuminating them with ultraviolet radiation.

In order to make these studies on living organs it was necessary to use a microscope whose construction differs greatly from the usual one in that use is made of reflected dark and light field illumination instead of transmitted light. The light field illumination is obtained by having a prism situated above the objective. The filtered radiation from a metal arc lamp is projected into the prism and is reflected through the objective to the object. Dark field illumination is obtained by directing the radiation outside the objective with the aid of two mirrors. Of these one is a plane mirror situated above the objective. Into this mirror the rays of the metal arc are projected and from these are reflected to a ring mirror which is placed around the front lens of the objective and from this mirror the light strikes the organ obliquely.

A water perfusion cap and a focusing device are connected with the objective. Saline immersion is necessary in order to avoid desiccation of the tissue, to reduce uneven reflection from the tissue, and to help increase the numerical aperture of the lens. A focusing device is essential to keep the object at a proper distance from the lens as otherwise capillary action would prevent focusing with high magnification.

The use of such a microscope permits the observation of cells in an undamaged condition. Upon the injection of fluorescent substances the normal appearance of the cells before exposing them to a long period of irradiation becomes that of brilliant transparency. It is possible to see cell boundaries and contents distinctly. After exposing the cells to irradiation for five or six hours, they gradually lose their brilliance and the cell structure becomes indistinguishable.

The length of time required for these changes to take place varies according to the substances injected. Fluorescent substances which have a strong photodynamic action show this effect earli-

er and to a greater extent than does aesculine which has little noticeable effect on the living cells. Different organs show these changes in varying degrees as I have described in my papers of 1933 and 1934. For example, the liver seems to be more sensitive than the kidney. The suprarenal gland showed not only a change in the density of the surface, but the surface became first uneven and puffed out like popcorn.

Undoubtedly these changes occur on the surface of the cells, as slides made from organs which showed these alterations in the living cell, showed no changes in the nuclei.

This seems to be in accordance with Blum's and Ponder's observations on the red blood corpuscles and thus I think we can state that cells in the living organism injected with photosensitizing substances and irradiated with ultraviolet light show increasing density on the surface.

Dr. Blum: Singer's experiments should emphasize the necessity of taking photodynamic effects into consideration when using such dyes in the study of living tissues.

Dr. Abramson: Bassen and I have been studying the effects of crude lecithin on human red blood cells in the anemias. We have observed that the same transformation to the spherical form that has been obtained for normal cells may be obtained with the cells showing anisocytosis and poikilocytosis. This offers a new method of obtaining information in regard to the actual changes in size in the anemias. Now Blum and Ponder offer a new technique which portends to be of even greater clinical application, for it will be now possible to study not only the size distribution of red cells in the anemias, but also to work with more clearly defined chemical systems.

Dr. Smetana: I have been interested in the problem of photodynamic action for some time and although my studies are not yet completed, I shall present some results here which are of interest with reference to Blum's presentation.

I am especially interested in finding out what goes on in the body of an animal after its sensitization and exposure to light. Since the methods applicable to living animals are too unreliable to touch the bottom of the problem, we had to resort to studying the photodynamic action of physiological substances *in vitro*. It is an established fact that photodynamic action is an oxidation process and therefore we chose the O_2 consumption as a measure to study the effect of light on sensitized physiological substances.

Experimental Conditions

All experiments were carried out with Fenn's

respirometer in a water bath at constant temperature.

Light source: A 500 watt lamp, the intensity of which could be controlled.

Sensitizer: Hematoporphyrin (Hp), Nencki, in a weak alkaline solution of pH 7.5.

Substrates: Blood constituents, lymph, urine, and uric acid.

Temperature: 37.5° C. unless otherwise stated.

Study of the Factors Influencing the O_2 Consumption of Sensitized Substrates During Exposure to Light

1. *Influence of light:* Analyses of the O_2 consumption of different sensitized media exposed to light of different intensities showed a linear relationship between intensity of light and O_2 consumption when all other factors were kept constant.

2. *Influence of dilutions of Hp:* The curves obtained by plotting the cmm. of O_2 consumed by different substrates during 10 minutes of exposure to light against dilutions of Hp ranging from 10^{-8} to 10^{-6} showed a steep incline of the O_2 uptake between the concentration of Hp 10^{-5} to about $10^{-4.8}$ and a more gradual slope from there to a concentration of 10^{-3} . Higher concentrations were not studied. In plotting the logarithms of these figures straight lines were obtained in all cases.

3. *Dilutions of substrates:* The curves obtained by plotting the O_2 consumption per cc. of various substrates against their dilutions ranging from 1/1 to 1/100, while all other factors were kept constant, were similar to those showing the effect of dilutions of Hp. Likewise, the plotting of the logarithms of the figures obtained resulted in straight lines.

4. *Influence of temperature:* The variation of the temperature of the water bath from 0° C. to 50° C., while all other factors were kept constant, had little influence on the O_2 consumption of the media studied: the O_2 uptake was smaller at a lower temperature, gradually rising to a maximum of about 25% at 40° C., and then slightly slowing down towards 50° C.

5. *Influence of pH:* Studies made with identical substrates of different pH values ranging from 7.5 to 9 showed an increase to a maximum of about 20% towards the greater pH.

6. *Influence of length of time of exposure to light:* The slowing down of the O_2 consumption of various sensitized media after prolonged exposure to light was found to be due to the oxidation of the substrate. Although the Hp itself undergoes a definite change during the exposure

to light, this change was found not to be responsible for the slowing down of the reaction.

Study of the O₂ Consumption of Constituents of Blood, Lymph, and Urine

Analyses of the data obtained by the exposure of the various constituents of blood (plasma, serum, albumin, globulin, fibrinogen, washed erythrocytes, ultrafiltrate of plasma, glucose, serum fats, as well as whole blood) to light in the presence of Hp showed that the plasma proteins

alone account for almost all the O₂ consumed during the experiment. Likewise, the protein content of lymph accounts for the O₂ consumption of this substrate during its exposure to light in the presence of Hp.

Similar experiments carried out with urine revealed that uric acid present in this medium is almost solely responsible for O₂ uptake during the experiment.

The experiments which at present are limited to body fluids only will be extended to cells of various organs along similar lines.

THE MULTIPLE NATURE OF VITAMIN D

CHARLES E. BILLS

In the development of knowledge of vitamin D there have been five particular discoveries which stood out from the painstaking investigations upon which they were based. First came the identification of vitamin D as a substance distinct from vitamin A by McCollum, Simmonds, Becker, and Shipley in 1922. Second was the discovery of activation—the observation that ultraviolet radiation endows foodstuffs with the antiricketic property exhibited by fish oils. This was the work of Hess and of Steenbock, and their associates, in 1924. Third was the finding that the acceptor of the ultraviolet rays in activation is the sterol fraction of the edible materials. This was the work of Hess (1925) and Steenbock (1925) in this country, and Rosenheim and Webster (1925) in England. Fourth was the identification of “ergosterol” as the parent substance of vitamin D. Many workers participated in this, but perhaps the names of Windaus and Hess (1927) and Rosenheim and Webster (1927) are the most prominent. The fifth major development was the isolation of calciferol, the active component of irradiated ergosterol, late in 1931. Details of the procedure of isolation, and the properties of the vitamin were published in 1932 by the English group headed by Bourdillon, and the German group of Windaus and Linsert.

The studies which led to the identification of “ergosterol” as the parent substance of vitamin D have already become a classic of chemistry. Even though they involve an extraordinary instance of mistaken identity, their practical importance is unimpaired and their history is none the less interesting. In briefest outline, these studies were as follows. Cholesterol of supposedly good purity was found to be activated by irradiation. Before irradiation, it exhibited spectral absorption in the ultraviolet region; after irradiation, it had little or no absorption. In consideration of Beer's law, one postulated that either the cholesterol had been at least half metamorphosed, or else the substance in which the absorption spectrum was changed was a small amount of impurity which was exceedingly absorptive. It was found that repeated crystallization of cholesterol led to the accumulation of the absorbing substance in the least soluble fraction. Furthermore, the use of very drastic means of purification, such as bromination followed by debromination, led to the production of a cholesterol which apparently had no absorption and no activatability. The drastic means included, in addition to bromine, such oxidizing agents as permanganate and decolorizing charcoal, and so it appeared that the unknown provitamin might be a

highly unsaturated sterol, such as ergosterol. Ergosterol was found to be destroyed by the same reagents which destroyed the X-substance in cholesterol. In one instance, namely in treatment with permanganate, even the rates of destruction of the provitamin and of ergosterol were found to be the same. Furthermore, the absorption spectrum of ergosterol was found to consist of four bands with maxima at 293.5, 282, 270, and 260 mμ, which are also the maxima exhibited by ordinary cholesterol. In ergosterol, the absorption was enormously more intense than in cholesterol, and the vitamin D potency after irradiation was enormously greater. Upon irradiation, the absorption bands of ergosterol underwent changes, and finally faded as did those of the impurity in cholesterol.

Such an identification of ergosterol as the provitamin D of cholesterol seemed excellent indeed, yet as Waddell demonstrated a year ago, it was erroneous. Not only is ergosterol different from the provitamin D of cholesterol, but the vitamin D produced by the irradiation of ergosterol is different from the vitamin D associated with cholesterol in fish oils. Until 1930, nearly all investigators assumed that these two forms of vitamin D were identical, and even now the existence of more than one form of vitamin D is not universally admitted. In recent months, the situation has become still more complicated by the discovery that more than one form exists in fish oils.

I am now going to consider, as nearly in chronological order as possible, the development of our knowledge of the multiple nature of vitamin D. This is an old and favorite interest of mine, for in 1927 I wrote, at the end of a paper on the distribution and origin of vitamin D, “It is not known whether the antiricketic substance of fish oils, of irradiated mammals, of animals that have eaten antiricketic foods, and of irradiated foods and their sterols, is one and the same substance. Quite possibly vitamin D is not a single substance, but a mixture or series of substances as variable as the sterols with which it seems to be almost inseparably associated.”

In 1926 I was attempting, in collaboration with McDonald, to activate cholesterol by means of catalysts. At this time—barely a year after the activation of cholesterol by irradiation had been announced—almost nothing was known of the mechanism of activation, and there was no evidence of the existence of any provitamin other than cholesterol. Everyone was therefore free to develop theories, however rash, in explanation of the formation of the vitamin. It so happened that at this time cholesterol was regarded as a terpene,

and it was known that ultraviolet irradiation induces polymerization in certain terpenes. We argued that if activation consists in polymerization, then the best polymerization catalyst should be the best activating agent. We therefore conducted experiments in treating cholesterol with fuller's earth—the catalyst which Gurvich had found effective in polymerizing pinene. Under proper conditions a pretty reaction occurred, and there was formed a substance of high molecular weight. A faulty molecular weight determination led us to regard this substance as a polymer of cholesterol, though we soon learned that it was dicholesteryl ether. Dicholesteryl ether had no antiricketic action, but a by-product of its formation, or, more properly, its degradation product, exhibited distinct antiricketic action.

The crude active substance was not remarkably potent, but weight for weight it had about the same activity as cod liver oil, and it was only slightly less potent than most preparations of irradiated cholesterol. Therefore it was intensely interesting at the time.

Before I undertook this experiment, I had been attempting to learn something of the chemical nature of vitamin D by investigating its stability toward various reagents. I had found that cod liver oil, and likewise a solution of irradiated cholesterol in oil lost all vitamin D activity when treated with butyl nitrite, and I had offered this fact as evidence that the two are identical. Now, therefore, McDonald and I applied the butyl nitrite test to the active product obtained from cholesterol by the action of fuller's earth. There resulted no decrease in antiricketic activity. Thus we obtained evidence of a very significant fact, namely that more than one molecular configuration in the sterols is capable of exhibiting antiricketic activity.

The formation of an antiricketic substance by the action of fuller's earth on cholesterol was confirmed by Kon, Daniels, and Steenbock (1928), and recently Yoder (1934, 1935) has advanced our knowledge of the mechanism of the reaction and of the identity of the active substance. Apparently the fuller's earth brings about a complete dehydration of the cholesterol, resulting in the formation of the doubly unsaturated hydrocarbon, cholesterilene. This becomes cholesterilene sulphonic acid, by reacting with the sulphur of the clay. According to Yoder, cholesterilene is inactive, but the vitamin D activity of cholesterilene sulphonic acid is measurable. It is distinctly greater than that of the crude fuller's earth reaction product. Nevertheless, it is only a few times greater than that of cod liver oil for the rat, though somewhat more so for the chicken. However, one must regard the vitamin D activity of cholesterilene sulphonic acid as purely a matter of theoretical interest.

At this point, it is well to recall the reason why all forms of vitamin D are considered to be sterols. They are either produced by activation of sterols, or they occur naturally in the sterol fraction of fats. Admittedly, there is no proof that the vitamin D of fish oils is a sterol, since it has never been isolated. But circumstantial evidence points to the sterols, and the chemical behavior of the fish oil vitamin, so far as it is known, is not unlike that of sterols. Let us therefore postpone for the moment our consideration of the vitamin D of fish oils and regard cholesterilene sulphonic acid as the simplest and also the weakest form of vitamin D.

There is a second form of vitamin D which is likewise a derivative of cholesterol. This is the substance which is produced by irradiating cholesterol that has been specially purified to free it from the usual provitamin.

A year after the discovery of the activatability of ordinary cholesterol and a year before the supposed identification of ergosterol as the provitamin to which this activatability is due, McDonald and I were investigating some properties of irradiated cholesterol (Bills, 1925). We took what we considered special pains to obtain pure cholesterol. Our crude cholesterol was repeatedly boiled in alcoholic solution with purifying charcoal, and the treated product recrystallized after each treatment. The cholesterol so prepared was readily activated by irradiation.

We were therefore more than ordinarily interested by the news that the English and German workers in 1926 were finding that, among other agents, charcoal was effective in purifying cholesterol so completely that it could not be activated. We then repeated the treatment of cholesterol with charcoal, subjecting the cholesterol to exceptionally long contact with it. We also subjected cholesterol to bromination and debromination, not once, but three times in succession. Ergosterol is instantly and completely destroyed by contact with bromine. All these preparations were activatable, and so also was a sample of allegedly non-activatable cholesterol which Windaus made and which we obtained through the kindness of Dr. Hess. It was noticeable, however, that the purified specimens were decidedly less activatable than the original material, yet when compared with each other, there was no discernible difference in the activatability of the several specimens. That is to say, a specimen thrice brominated was not less activatable than a specimen once brominated, or than the specimen drastically treated with charcoal (Bills, Honeywell, and MacNair, 1928).

Jendrassik and Keményfi (1927), in Hungary, had reported that cholesterol purified by bromine always retained a fraction of its activatability. To explain this, they postulated the existence,

under suitable conditions, of an equilibrium between cholesterol and provitamin. Somewhat inconsistently with this explanation, they reported that cholesterol treated with charcoal was not activatable.

Kon, Daniels, and Steenbock (1928) confirmed our observation that cholesterol, purified with either charcoal or bromine, is activatable. They claimed, however, that permanganate gave a cholesterol devoid of activatability. Disregarding the known sensitiveness of ergosterol to bromine, they concluded that ergosterol was the source of the activatability in the preparations treated with bromine, as well as in those treated with charcoal.

With a background of some years, it now appears that a reconciliation of these seemingly conflicting claims is not impossible. The facts of the matter probably are (1) that cholesterol which is sufficiently pure is not activatable, (2) that ergosterol is not the provitamin in highly purified cholesterol, and (3) that the real nature of this provitamin is still unknown.

With the cooperation of Dr. MacNair of the Bureau of Standards the samples of specially purified cholesterol which Miss Honeywell and I had prepared were studied spectrographically. Under the ordinary conditions of spectrographic examination they showed no absorption of ultraviolet light, and to this extent were in keeping with the described products of the European investigators. Yet it seemed evident that inasmuch as these preparations were activatable they must show somewhere the absorption of the activating rays. We therefore studied the absorption in thicker layers of stronger solutions. A spectrogram taken through 20 cm. of a 15 per cent solution in ether showed five faint absorption bands with maxima at 315, 304, 293.5, 282, and 269 $m\mu$. The last three were apparently identical with three of the ergosterol bands. The general absorption of this thick layer of concentrated solution was so great at 260 $m\mu$ and below that we could not ascertain whether the fourth ergosterol band, which we had just discovered in this position, was present.

Quantitatively, the three bands with maxima at 269, 282, and 293.5 $m\mu$ were only about 1/150 as intense as in the original unpurified cholesterol. Nevertheless, the purified product was 1/30 as activatable as the unpurified. From this not wholly justifiable biological comparison, and particularly from the presence of the two additional bands with maxima at 304 and 315 $m\mu$, which ergosterol does not exhibit, we concluded that the activatability of the specially treated cholesterol was due either to cholesterol itself or to a hitherto undiscovered impurity which persisted after three successive treatments with bromine.

We were inclined to associate the activatability of the purified cholesterol with the absorption bands at 315 and 304 $m\mu$, rather than with the shorter wavelength bands. Heilbron, Morton, and Sexton (1928), however, called attention to the fact that the bands at 315 and 304 $m\mu$, and also the band at 293.5 $m\mu$, might well be those of cholesterilene, which quite conceivably was formed in the harsh treatment to which the cholesterol was subjected. They reported that cholesterilene was not rendered active by irradiation.

Next to take up the study of purified cholesterol were Koch, Koch, and Ragins in 1929. These workers again found that cholesterol purified by bromine was activatable. So also was a specimen treated with permanganate. They made the noteworthy contribution that the slight activatability of the treated cholesterol preparations was increased 25-fold by heating the sterol after purification. Koch, Koch, and Lemon (1929) associated the activatability of the heated cholesterol with a strong general absorption in the ultraviolet region, rather than with any specific banded absorption. It is clear from their work that the provitamin D in the heated cholesterol was mainly, if not entirely, a transformation product of cholesterol itself, and not any contaminant in the original crude cholesterol which escaped destruction.

Schoenheimer (1931) applied to Koch's experiments the interpretation that cholesterol, when heated, undergoes a simultaneous hydrogenation-dehydrogenation reaction, resulting in the formation of a completely saturated sterol and a highly unsaturated, ergosterol-like body which is activatable. This interpretation is, in fact, an application to an *in vitro* reaction of the hypothesis offered by Heilbron and Sexton (1929) and by Schoenheimer to explain the existence in plants and animals of sterols with different degrees of unsaturation.

One should recall that the analytical determinations of Windaus, v. Werder, and Gschaider (1932) and Windaus and Lüttringhaus (1932) established with a fair degree of certainty that ergosterol has 28 atoms of carbon, whereas cholesterol has but 27. The provitamin in Koch's preparations therefore could not be ergosterol, although it might possibly be a lower homolog of ergosterol. Apparently in the act of regenerating cholesterol from its dibromide, or of treating it with mild oxidizing agents such as charcoal and permanganate, one produces a provitamin of fewer carbon atoms than ergosterol. Something of a similar nature must occur when the purified cholesterol is heated.

At first thought it would seem that the provitamin which is formed by heating purified cholesterol might be identical with the one which in smaller quantity, is present in cholesterol

chemically purified. (The differences in absorption spectra might be due merely to the presence of interfering substances in the heated product). Nevertheless, it seems that the provitamins are actually different. This is revealed in unpublished work by Dr. Millicent Hathaway of the University of Illinois. She has recently found that in tests with chickens the vitamin D produced by irradiating heated purified cholesterol is more effective, rat unit for rat unit, than the vitamin D produced by irradiating non-heated purified cholesterol. The former behaves like cod liver oil, the latter like calciferol. Thus Hathaway's vitamin D, prepared from Koch's provitamin D, is distinguished as a third form of this vitamin.

Although our knowledge of the second and third forms of vitamin D is exceedingly limited, we can infer from the fact that the provitamins are not produced in abundance, that the vitamins themselves, were it possible to obtain them in concentrated form or to isolate them, would be found to possess a high degree of physiological activity. I would caution you against regarding these forms as merely laboratory curiosities, like cholesterol sulphonic acid. It is conceivable that they are important in natural products, such as the fish oils.

We shall now pass to the consideration of the experiments which demonstrated that the vitamin D of irradiated ergosterol is a substance distinct from the vitamin D of cod liver oil. When irradiated ergosterol was discovered, nearly everyone who knew about it believed that it was the same vitamin D which, in great dilution, was the active principle in cod liver oil. In 1928 a request came to our laboratory from Dr. Carrick of Purdue University, to supply a sample of standardized solution of irradiated ergosterol. Carrick wanted to use this newly available commercial product as a substitute for cod liver oil in some rations for chickens with which he was working at the Indiana experiment station. I sent him an oily solution which had been standardized to be 100 times as potent as average cod liver oil for healing rickets in rats. After a time Carrick wrote that the solution failed to exert the expected protective action in chickens. A second solution was supplied, but the results were no better. I obtained an unused portion of this solution, and reassayed it with rats, finding it to be just as effective with this species as it had been originally. Thereupon Dr. Massengale of our laboratory carried out a systematic investigation of the responses of both rats and chickens, which was published in 1930. This study showed plainly that, rat unit for rat unit, the vitamin D of irradiated ergosterol was about 100 times less effective for chickens than the vitamin D of cod liver oil.

A few weeks before Massengale's paper was printed, results of a similar nature were briefly reported by Mussehl and Ackerson (1930) of the Nebraska experiment station, and by Hess and Supplee (1930) of New York. There have since been a large number of studies which establish beyond reasonable doubt that these two important sources of vitamin D are physiologically different. Such studies have made it plain that alternative explanations of the anomaly are untenable. Thus, for example, the difference in effectiveness of the two forms is not due to the presence or absence of vitamin A, or to the nature of the oily vehicle in which the vitamin is held.

There are several other physiological effects by which the chemical difference between these two forms of vitamin D is revealed. One is that irradiated ergosterol tends to elevate the blood phosphorus of an animal relatively more than cod liver oil when the same degree of protection against rickets is conferred. Another is that the response curves of a species such as the chicken have a different shape for each of these two vitamin sources. By response curves, I mean the curves which one obtains when one constructs a graph correlating the percentage of bone ash with the vitamin unitage administered. Dr. Massengale and I have lately been constructing these curves as the basis of a highly accurate method for the quantitative estimation of vitamin D with the chicken. The statement which I made that a rat unit of one form of vitamin D is 100 times more effective than a rat unit of another form is literally true for only one point on the response curve. Actually the difference varies from zero to infinity—zero when the response is infinitely small, and infinity when the response is very large. Unless the test period is extended to longer than four weeks, one cannot produce quite as hard a bone in chickens with irradiated ergosterol as one can with cod liver oil, no matter how large the dosage given. The difference of 100 times is found when a bone ash percentage of about 47 is produced in young Leghorn chicks in four weeks of treatment.

The practical aspects of this discovery are so frequently misinterpreted that I am taking this opportunity to call attention to the salient points. The most obvious point is that irradiated ergosterol is of little value to the raiser of poultry. Even if the patent monopoly on its manufacture did not exist, this form of vitamin D would still be uneconomical in comparison with cod liver oil in the feeding of chickens.

The situation as regards the human infant is utterly different from that with the chicken. Reviewing this field, I recently wrote (Bills, 1935) as follows, "The human being comes between the chicken and the rat, but nearer the rat, in response to the two forms of vitamin D. It is

noteworthy that, in spite of the almost immediate adoption of irradiated ergosterol as a therapeutic agent, nearly three years passed before evidence was obtained that a rat unit of this form of vitamin D exerts on the infant a quantitatively different effect than a rat unit of cod liver oil. While this is no compliment to the preciseness of clinical medicine, it is doubtless explained in part by the fact that precise methods of standardization with rats were not in general use for either cod liver oil or activated ergosterol. Evidence that cod liver oil and irradiated ergosterol (Viosterol) in amounts equipotent for rats do not exert the same degree of antiricketic effect on children, came largely from the studies of Hess, Lewis, and Rivkin (1930) and Hess and Lewis (1932, 1933). These workers found that about 3 rat units of the ergosterol preparation were clinically equivalent to 1 rat unit of cod liver oil. The satisfactory experience of physicians with activated ergosterol, in the face of this anomaly, was attributed to the fact that the customary dose of Viosterol is ample to counterbalance the discrepancy in rat-unit effectiveness."

When I wrote these sentences, a year ago, I was aware that certain clinical workers had claimed much greater differences than Hess and Lewis. Others maintained that there was no appreciable difference, rat unit for rat unit, between irradiated ergosterol and cod liver oil as therapeutic agents.

A contributory factor to the belief that irradiated ergosterol was clinically inferior to cod liver oil, rat unit for rat unit, was the ill-chosen potency basis which until recently was imposed upon manufacturers by the Wisconsin Alumni Research Foundation (the holders of the patents on irradiation) and by the Council on Pharmacy and Chemistry of the American Medical Association. On this basis, the potency of solutions of "Viosterol" was designated "100 D" or "250 D" when the solutions were 100, or 250, times as potent in vitamin D as a hypothetical cod liver oil which contained approximately 36 per cent as many rat units of vitamin D per gram as cod liver oil of average potency. Potencies are now expressed in U.S.P. units, which are nominally identical with the international units adopted by the Health Organisation of the League of Nations.

Another factor was the poor technical quality of nearly all studies in which children served as the test animals on one side of the comparison. The studies recently reported by Eliot (1935) are therefore particularly significant, in that they involved the use, not only of carefully standardized materials, but fairly generous numbers of selected children. Eliot and her associates were unable to detect any significant difference, rat unit for

rat unit, between irradiated ergosterol and cod liver oil as antiricketic agents for children.

The unqualified statement that one of these forms of vitamin D is more, or less, effective than the other, is never logically permissible. The logical statement of the known facts requires a few more words, and can be made in either of two ways: First, that irradiated ergosterol is less effective, rat unit for rat unit, than cod liver oil for the chicken; second, that irradiated ergosterol is more effective, chicken unit for chicken unit, than cod liver oil for the rat. To the uncritical ear, these two expressions sound contradictory, yet their meanings are identical. I emphasize this point because I have noticed confusion over it many times in the medical literature.

The active principle in irradiated ergosterol has been isolated and is known by the chemical name, calciferol. This form of vitamin D has been the subject of many studies, both physiological and chemical. Like irradiated ergosterol, it is inferior, rat unit for rat unit, to cod liver oil for the chicken. We have found that the degree of its inferiority to cod liver oil, rat unit for rat unit, is the same as that of crude irradiated ergosterol. Its absolute potency, for the rat, is enormous, being about 400,000 times that of average cod liver oil. It is thus the most powerful antiricketic agent known, although conceivably some of the forms of vitamin D which occur in fish oils might, in the isolated state, be equally or even more potent.

Chemically, calciferol is an isomer of ergosterol, having the formula, $C_{28}H_{48}OH$. Thus it differs from the hydrocarbon, cholesterolene, $C_{27}H_{44}$, and from any vitamin D that can arise, like Koch's vitamin D, from the isomerization or breaking down of cholesterol, $C_{27}H_{46}OH$. Calciferol is thus to be regarded as the fourth form of vitamin D.

In the course of our studies on activation, we have made many attempts to produce vitamin D by other means than irradiation. In recent years most of these efforts have been directed at the isomerization of ergosterol. In 1931 I reported with McDonald the results of a series of experiments in which a slight measure of success was attained. The product, however, was no more than another laboratory curiosity.

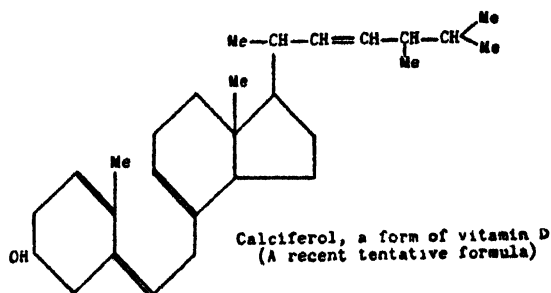
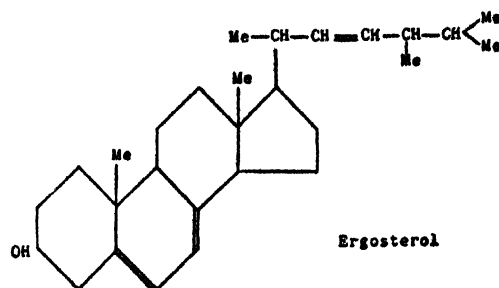
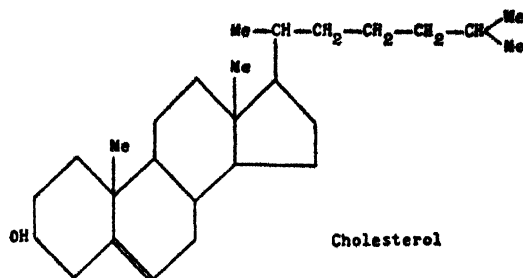
We treated ergosterol with alkyl nitrites, such as ethyl nitrite, or with nitrous fumes, and then treated the reaction product with alkyl amines, such as isopropylamine. There resulted a product which, for the rat, had several times greater antiricketic activity than cod liver oil. We did not succeed in separating the active principle from its by-products, and consequently we can make no statement in regard to the potency of this form of vitamin D in the pure state. However, it will

suffice to illustrate our thesis on the multiple nature of vitamin D by calling attention to the fact that this fifth form of the vitamin was produced by the action of the same reagents, nitrites, which destroyed the vitamins D in cod liver oil and irradiated cholesterol. As an ergosterol derivative, it presumably contains one atom more of carbon than any vitamin D derived from cholesterol, and as a substance which apparently contains nitrogen, it differs from any form of vitamin D derived from either cholesterol or ergosterol. So again we have a form of vitamin D, of no practical importance because of its low activity, but theoretically significant to the chemist.

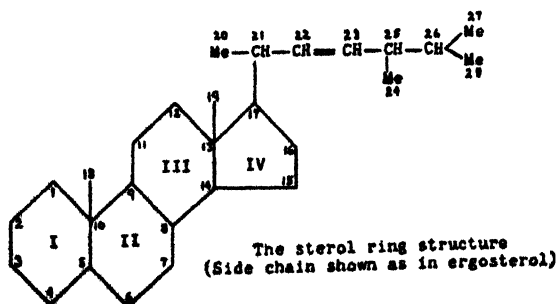
There is a sixth form of vitamin D, discovered in 1933, which, like two or three of the others described above, is of no practical importance unless it should turn out to be one of the forms naturally occurring in fish oils. This form has unusual theoretical interest because its chemistry is so well known. For this reason I shall describe its preparation in some detail.

As a result of the brilliant investigations on the constitution of the sterols which are associated with the names of Windaus, Wieland, Rosenheim, Heilbron, and Bernal and their associates, it has become known in the past few years that cholesterol and ergosterol, and in fact all sterols, are built around the skeleton of a hypothetical hydrocarbon called cholane, or phenanthrene-cyclopentane (Bills, 1935). In ergosterol and cholesterol there is a long open side chain attached to the cyclopentane ring. Ergosterol contains three double bonds, one of which is in the side chain at carbon atom No. 22, and two of which are in the phenanthrene ring. It is known that in activation there is a shifting of the double bonds in the ring, accompanied by a rupture of the phenanthrene ring and the formation, by this rupture, of a fourth double bond.

Windaus and Langer (1933), at Goettingen, made a study which revealed the importance of the three double bonds of ergosterol in activation. By heating ergosteryl acetate with maleic anhydride, they produced the addition product, ergosteryl acetate-maleic anhydride, in which the two



double bonds in the ring are saturated. With this part of the molecule temporarily protected, they hydrogenated the double bond at position 22 in the side chain. The reduction product was then thermally decomposed, splitting off the maleic anhydride and giving the acetate of 22-dihydroergosterol in which the two double bonds in the ring were restored. From this the free sterol was prepared. The German workers described 22-dihydroergosterol as exhibiting the same spectral absorption as ergosterol. They found that it became active upon irradiation, although less so than ergosterol. The vitamin produced by the irradiation of 22-dihydroergosterol has not been isolated, but it is probably 22-dihydrocalciferol, which contains 2 more atoms of hydrogen than calciferol or any other ergosterol isomer, and 1 more atom of carbon than any isomer of cholesterol.



Up to this point we have seen that there are six chemically distinct sterol derivatives which exhibit in greater or lesser degree the calcification-promoting property of vitamin D. These are, if you please to so regard them, six vitamins

D. But we have not yet considered the vitamins D of irradiated foods and of fish oils.

So far as anyone knows, nothing but sterols can be endowed with antiricketic properties by irradiation. It is universally held that the activation of foods is actually the activation of the sterols which they contain. On this basis one postulates at the beginning of the discussion that there can be formed in irradiated foods as many kinds of vitamin D as there are kinds of sterols in the foodstuff that are capable of being activated. This does not solve our problem, but merely complicates it, because until recently it was so generally believed that ergosterol is the only important activatable sterol that research on other provitamins D was neglected.

According to Gérard's rule of forty years standing, ergosterol is the principal sterol of fungi, phytosterol of the higher plants, and cholesterol of animals (Bills, 1935). It was thought a few years ago that there was an exception to the rule, in that ergosterol, in traces, occurred admixed with all natural sterols, and upon this idea were built all the intriguing hypotheses concerning the origin of vitamin D in the skin of animals exposed to sunlight. Everything was simple until Waddell demonstrated that the provitamin D of spinal cord cholesterol is not ergosterol. Now we find ourselves as of 1926, except that our background for future studies has improved.

Let us consider first the fungi. Yeast is the only fungus which is irradiated commercially, but the quantities of it which are so treated are so large that yeast is one of the two most important foodstuffs in which vitamin D is built up. The principal field of distribution for vitamin D in irradiated yeast is not the familiar foil package, as you might guess, but the stock yeast which is fed to cattle for the production of the so-called yeast milk.

The principal sterol of yeast is ergosterol. As a matter of fact, yeast contains so much ergosterol—up to 2 per cent of its dry weight, according to species and conditions of culture—that the ergosterol of commerce is largely made from this fungus. Thus there is good reason to believe that the vitamin D which is formed in the irradiation of yeast is calciferol. Experimental evidence for this assumption is found in the work of Mussehl and Ackerson (1930), who established that irradiated yeast is less effective, rat unit for rat unit, than cod liver oil for the chicken. This was confirmed by Steenbock, Kletzien, and Halpin (1932) and Bethke, Record, and Kennard (1933), who showed that the difference is a large one, comparable with the difference observed between irradiated ergosterol and cod liver oil. The kind of vitamin D present in irradiated yeast is

evidenced even in the milk obtained from cattle which have eaten it. Krauss, Bethke, and Monroe (1932) found that the butterfat from yeast milk was less than one-fourth as effective, rat unit for rat unit, as cod liver oil for preventing leg weakness in chickens. Work recently announced by Bethke, Krauss, Record, and Wilder (1935) and by Haman and Steenbock (1935) indicates that the difference in effectiveness, per rat unit, is about 10 times under the experimental conditions established by these investigators.

When we leave the fungi and consider the higher plants, we encounter the fact that the phytosterol of the higher plants is no single substance. It is merely a mixture of phyto-sterols, of which there are many. Stigmasterol, sitosterol, and dihydrositosterol have been reported to acquire no antiricketic property by irradiation when they are sufficiently pure, but in view of the history of cholesterol I feel that the first two deserve reinvestigation. Specimens of sitosterol of ordinary purity have repeatedly been found to exhibit the "ergosterol" absorption bands and to be activatable. Their spectral absorption is generally several times more intense than that of ordinary cholesterol, and, as in the case of cholesterol, it is practically lost upon drastic purification. Some of the numerous phytosterols have never been investigated from the standpoint of activation. Whether the "ergosterol" absorption bands in the vegetable sterols are really due to ergosterol I cannot say at the present time. This question is now under investigation in our laboratory and in at least one other laboratory. The method of study is, of course, the use of the rat and the chicken, which method has become our principal tool of progress in a field where the ordinary methods of chemistry fall down and spectrographic methods are misleading. The mere fact that the spectral absorption bands seen in the vegetable sterols look like those of ergosterol is no proof that ergosterol is present, for the same series of bands is shown by 22-dihydroergosterol and by the provitamin D in cholesterol which Waddell has studied in work which I shall presently describe.

Waddell's interest in the provitamin D of cholesterol, and broadly, in the provitamin D of animal sources, came about through the fact that his firm had acquired the patent rights to irradiate ergosterol for poultry use just prior to the discovery that irradiated ergosterol was comparatively useless for this purpose. In seeking a solution of his problem, his attention was attracted by certain facts which were not wholly in keeping with the supposed identification of ergosterol as the provitamin D of cholesterol. Among these was the noteworthy efficacy, for the prevention of rickets in chickens, of short direct exposures

to ultraviolet rays of low intensity such as occur in winter sunshine, in contrast to the inefficacy of irradiated ergosterol. And again, there was the fact of the non-absorbability of unirradiated ergosterol by most animals, which marked it as a sterol foreign to the animal body.

Waddell (1934) demonstrated that cholesterol containing the usual provitamin gives upon irradiation a form of vitamin D which is at least as effective, rat unit for rat unit, as cod liver oil for the chicken. He found, moreover, that ergosterol irradiated in the presence of cholesterol is no more effective than ergosterol irradiated by itself. Therefore the provitamin D of ordinary cholesterol is not ergosterol, and the vitamin D produced by irradiating ordinary cholesterol is not calciferol.

The possibilities opened up by this work are immense. Quantitative bioassays with rats and chickens are needed to show whether Waddell's vitamin D (irradiated ordinary cholesterol) is distinguishable from the Koch-Hathaway vitamin D (irradiated heated purified cholesterol) or from the Windaus-Langer vitamin D (irradiated 22-dihydroergosterol). It cannot be identical with any of the other four forms, for reasons expounded earlier in our argument. It may be a seventh form of vitamin D.

In a lecture delivered a few months before Waddell's paper appeared, Callow (1934), speaking for the English team of investigators with calciferol, admitted the possibility that the vitamin D of cod liver oil may be different from calciferol. He suggested that, in view of the work on 22-dihydroergosterol, the "natural provitamin is a cholesterol derivative with the double bonds in the critical position, a demethyl-dihydroergosterol, and that natural vitamin D is a demethyl-dihydrocalciferol." It occurs to me that this suggestion can be applied extraordinarily well to Waddell's vitamin D in at least three respects.

First, the hypothetical demethyl-dihydroergosterol, or, as it might be called, 7-dehydrocholesterol, would be a substance identical with ergosterol except in the side chain. It is known that the spectral absorption bands of ergosterol are due to the two double bonds in the phenanthrene ring. Therefore, the hypothetical substance should exhibit the same bands as ergosterol and 22-dihydroergosterol. The bands of the provitamin are actually very similar to, and seemingly indistinguishable from these.

Second, it is obvious that 7-dehydrocholesterol, lacking the side chain methyl group of ergosterol, is a C_{27} compound, rather than a C_{28} compound. Although it is not true that any sterol with 27 carbon atoms can be absorbed by the animal body, it does seem to be the rule, with exceptions, that animal sterols have one fewer carbon atoms than the common sterols of the vegetable kingdom.

Third, it is to be expected that 7-dehydrocholesterol, lacking the double bond of the ergosterol side chain, would be a more stable substance than ergosterol. Years ago I commented on the anomaly that ergosterol, so unstable by itself, should withstand years of aging as the provitamin D of cholesterol. I had detected the "ergosterol" absorption bands in a sample of gallstone cholesterol sixteen years old. King, Rosenheim, and Webster (1929) made the same observation, *a fortiori*, on the sterols from the brain of a Coptic mummy.

The cholesterol used in Waddell's experiments was obtained from the laboratory of a meat-packing firm which makes it, so I am told, from spinal cords. While it is impossible to say that cholesterol from all sources carry the same provitamin D as spinal cord cholesterol, the assumption is that they do. We have under investigation by the rat-and-chicken method cholesterol from spinal cord in comparison with that from halibut liver oil, human skin, and butterfat, each of which has an obvious significance.

In April of this year two reports were made on the efficacy of irradiated milk, one by Bethke, Krauss, Record, and Wilder (1935) and the other by Haman and Steenbock (1935). These workers agreed that, rat unit for rat unit, the vitamin D of irradiated milk is of the same order of effectiveness for the chicken as the vitamin D of cod liver oil, and ten times as effective as that of yeast milk. Haman and Steenbock found no difference in effectiveness between irradiated milk and irradiated cholesterol. Thus there is evidence that the provitamin D of milk cholesterol is the same as that of cord cholesterol, and consequently that the vitamin D of the two is the same, though different from that of irradiated ergosterol. This may be the beginning of a generalization in support of the view that the principal provitamin D of all cholesterol is the same.

We shall consider, finally, the vitamins D of fish oils. During the past several years we have extracted the liver oils of more than 100 species of fish, and have quantitatively assayed each for its content of vitamin A and vitamin D. The first assays for vitamin D were done in every instance with rats, of which we used in all about ten thousand. This work revealed enormous species differences in vitamin D potency. Two of the oils contained less than 1 international unit of vitamin D per gram, and several contained more than 60,000 units per gram. Cod liver oil, by way of comparison, contains on the average 100 units per gram, and is one of the weaker sources.

Although it has not been the custom to question the singleness of "natural" vitamin D, we considered that of all sources where different forms might naturally occur, these vastly different fish

oils were the most likely. We therefore assayed twenty-five of the oils quantitatively with chickens, and reassayed these chosen specimens with an extra number of rats. With the methods employed, the probable error of our assays was only about 10 per cent of the unitage found.

The first special oil examined was halibut liver oil. Rat unit for rat unit, this was slightly less effective than cod liver oil for chickens, but the difference was not much more than the probable error of the assays, and certainly not more than the expected occasional error. Halibut liver oil contains on the average about 12 times as much vitamin D per gram as cod liver oil, and is thus an oil of medium potency.

Our next experiment was with a tuna liver oil, which on the average is 400 times as potent as cod liver oil when assayed with rats. This oil was found to be only one-sixth as potent, rat unit for rat unit, as cod liver oil for the chicken. Since it is known that certain esters of calciferol do not exert their antiricketic action until they have been hydrolyzed, it occurred to us that the strange behavior of the tuna liver oil might be due to the existence of the vitamin D in that oil as an ester which is easily hydrolyzable by the rat but not by the chicken. We therefore proceeded to saponify both oils, and to administer the unsaponifiable fractions to both chickens and rats. With both oils, the unsaponifiable fraction was somewhat more effective, rat unit for rat unit, than the oils themselves for the chicken. The enhancement of effectiveness amounted to 19 per cent in the tuna liver oil, and 37 per cent in the cod liver oil. These enhancements are sufficiently great to indicate strongly, though not beyond doubt, that in each oil at least part of the vitamin D had originally existed in a combination. Nevertheless, the unsaponifiable fraction of the tuna liver oil was only one-seventh as effective, rat unit for rat unit, as the unsaponifiable fraction of the cod liver oil for the chicken. The obvious explanation is that the vitamin D of this tuna liver oil and the vitamin D of cod liver oil are different substances or different mixtures of substances.

We continued the study of fish oils until we had investigated 25 species. The work I have mentioned here was briefly announced by Bills, Massengale, and Imboden (1934). The detailed study will be published next winter, or as soon as we have cleared up some straggling points. It is sufficient to say now, that in this extensive collection of oils we found some which, rat unit for rat unit, were for the chicken more effective than cod liver oil, and some which were less effective than the tuna liver oil. It is significant that when the scatter of the assays of these oils is recorded graphically, it reveals no marked concentration of samples around cod liver oil. If

it were otherwise, i.e., if there were more and more oils closely similar to cod liver oil, and fewer and fewer removed from it as regards the efficacy ratio for chickens and rats, then one would infer either that some regular source of error was at work, or that cod liver oil represented one single form of vitamin D. As it is, the scatter does not suggest any errors of unusual magnitude. It does indicate that cod liver oil vitamin D is just a mixture of forms of this vitamin, such as occurs, in different proportions and concentrations, in any of the other fish oils. At least, this must be one's interpretation of the observations, unless one makes the highly unreasonable assumption that each fish oil contains its own private form of the vitamin. In our estimation, therefore, the fact that irradiated cholesterol happens to exhibit, rat unit for rat unit, about the same effectiveness as cod liver oil for chickens, is no proof and is hardly even evidence that the vitamin D which arises from Waddell's provitamin D is the same as that naturally occurring in cod liver oil.

At present we do not know, and we may never know how many forms of vitamin D exist in fish oils. The first form of vitamin D that I reviewed, cholesterilene sulphonic acid, is certainly not in them, for it is not potent enough to account for the activity of even a mediocre oil. The fifth form—that which results from the action of alkyl nitrites on ergosterol—cannot be the form in cod liver oil, because the vitamin D of cod liver oil is destroyed by nitrites. The fourth form, calciferol, may occur in fish oils, but if it does it is not alone, for no fish oil that we have examined behaves as badly, rat unit for rat unit, as calciferol in the chicken. Ender, in 1933, made from tuna liver oil a vitamin D concentrate which was one-fourth as potent as calciferol for rats. It did not show the expected calciferol absorption band, and its rate of esterification with phthalic anhydride was much greater than that of calciferol. For these reasons, this particular tuna liver oil vitamin D cannot consist largely of calciferol. The second and third forms, those which are made in the laboratory by the irradiation of specially purified cholesterol or of Koch's provitamin, have not been obtained in concentrated state. Conceivably they are highly potent substances which could be factors in fish oils. The sixth form, that which comes from 22-dihydroergosterol, may be present. We can say more about this when our present experiments with chickens come through and possibly identify this with, or distinguish it from, the vitamin D produced by irradiating Waddell's provitamin. The hypothetical demethylhydrocalciferol is always something to be looked for in fish oils, and we cannot forget that there are still other possibilities among the isomers of chole-

terol and ergosterol, of which several hundred can theoretically exist.

Several topics which I have mentioned here only briefly are discussed more at length in my review of the sterols (Bills, 1935). I have tried today to expand only one subject and to search out its possibilities as far as I dared. If I have gone too far with the theoretical, it is because I feel that an informal review should not only consolidate one's thoughts on a subject, but open the way to new ideas.

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DISCUSSION

Dr. Bergmann: Until quite recently the organic chemist was inclined to conclude from a similarity in physiological behavior of two unknown substances that if not identical they were at least very closely chemically related. That such conclusions might lead to serious mistakes has been shown by Fritz Koezl in his excellent studies on the growth promoting substances of plants. He found that the original growth hormone, auxine, which is present in seedling tips, is a cyclopentene derivative, while the hormone found in the urine is partly, and in micro-organisms is exclusively the "heteroauxine" which is indolacetic acid. These two substances which are chemically absolutely different can replace each other in every known reaction on young oat or corn seedlings. They show only a slight difference in activity.

I think that Koezl's experience can teach every chemist who is engaged in the study and isolation of hitherto unknown highly potent substances an important lesson. As Bills has outlined, the chemists have for some time concluded that ergosterol was the provitamin D and calciferol the vitamin D. These conclusions were based on similarities in physiological behavior. They are as we know now incorrect and misleading though they have been doubtless very useful. I agree with Bills in believing that what I might call the artificial vitamins D, that is to say those which have been prepared from sterol derivatives either by irradiation or chemical means have certain chemical properties in common, for instance, the position of the double bonds. Whether the naturally occurring vitamins D (and thereby I am thinking especially of those occurring in fish oil) resemble the "artificial" substances at all, remains to be seen. There might be some evidence that they do but I think that under all circumstances one should keep one's mind free that they might not.

There is the question of the photochemical origin of the fish oil vitamin D which has always puzzled me. Obviously under the influence of irradiation experiments on sterols the theory has been elaborated that the sterols of algae or small plants are transformed by light into vitamin D, and that these algae are eaten by small fish which then are devoured by the bigger fish such as codfish, which then store the vitamin in the liver. I should like to ask Dr. Bills whether in his opinion this theory has any other foundations than the conclusions derived from the aforementioned irradiation experiments. Is it not likely that the presence of vitamin D in fish might have nothing at all to do with irradiation?

I want to mention again Koezl's experiences with the auxines. At the present time plant physiologists cannot offer the slightest explanation for the fact that the real auxine and the indolyl acetic acid show identical physiological behavior. It seemed for some time that the indolyl acetic acid promoted growth by what Koezl calls "using the back door". It was believed that indolyl acetic acid increased the rate of respiration in growing plants, thereby stimulating the metabolism in general and indirectly promoting growth. As interesting as this explanation was, it did not stand up under further experiments. I have mentioned this example because it seems to me that the ways of attack on the artificial and natural vitamins D are somewhat different. The artificial vitamins D might use "the back door" and in cases where there is no back door, as let us say in chickens, they are relatively ineffective. A closer study of the toxicity of the different vitamins D might throw some light on this problem.

I have presented these thoughts, the speculative character of which is quite clear to me, not without much hesitation. That I did put them down at all was mainly due to the encouraging sentence with which Bills closes his paper, namely: "An informal review should not only consolidate one's thoughts on a subject, but open the way to new ideas."

Dr. Bills: The theory which Bergmann mentioned in regard to the origin of vitamin D in fish has little to support it. Schmidt-Nielsen observed that the basking shark, which exposes itself to the sun for hours at a time and feeds on the plankton at the surface of the ocean, has a low concentration of vitamin D in its liver oil. Examinations of plankton have shown little, if any, vitamin D to be present. We found that capelin, the principal fish eaten by the Newfoundland cod during the fattening period, is a poor source of vitamin D. We also found that young catfish, grown in captivity on a vitamin D-free diet, exhibited the normal amount of vitamin D in their tissues. This was true whether they grew in the dark or were exposed to ultraviolet irradiation. It is my opinion, from this admittedly inconclusive evidence, that some fish, at least, have the power of synthesizing vitamin D. The larger fish, no doubt, get some vitamin D from their prey, but that is beside the point.

Dr. Harris: You mentioned that it is quite likely that in liver oils of fishes there is always more than one form of vitamin D present in varying absolute or relative amounts, which would account for the wide range of potency found. Have assays been made on liver oils of lower forms of life? One might guess that forms would be found in the evolutionary scale in which only one type of vitamin D is present; that the situation in the fishes represents a rather advanced evolutionary state in regard to vitamin D.

Dr. Bills: Unfortunately, the liver oils of the lower "fishes", the elasmobranchs and cyclostomes, contain very little vitamin D. The livers, or organs homologous with liver, of still lower forms have not been studied. The body oils of some lower forms such as shrimp, squid, and oysters, also contain only a little vitamin D. Cholesterol is almost universally present in the animal kingdom; it may be that some forms of vitamin D in small amounts are also widely distributed.

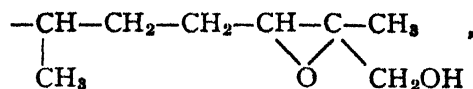
Dr. Ponder: Has the idea of using two species in vitamin assays been applied to distinguish new forms of vitamins other than D?

Dr. Bills: Some of the components of the vitamin B complex have been studied this way, but the possibilities of the method are far from exhausted.

Dr. Strain: One of the points that occurred to me during the address is that of the classifica-

tion of calciferol, dihydrocalciferol and perhaps the other compounds with vitamin D potency. Calciferol and dihydrocalciferol lack the perhydrocyclopentaphenanthrene nucleus characteristic of the sterols and probably should be classified not as sterols but as derivatives or degradation products. Of course the point is purely academic.

With respect to the differences in potency of the various fish oils two views are possible. Either the compounds responsible for the potency are quite similar, or as intimated by Bergmann, wholly different. Assuming that they are similar one might expect the differences in potency to be due to variations in the nature of the side chains. Unfortunately the chemistry of the liver of fish has not been well investigated. Bile fistulas cannot be made as in the case of dogs and other higher animals and in general the bile has not been examined. The shark, however, is an exception. In the bile of this fish the sulfuric acid ester of scymnol occurs in place of the usual bile salts. Scymnol differs from cholic acid in that the side chain has the structure,



and one of the hydroxyl groups occupies a different, as yet undetermined, position. Should this type of side chain be present in a compound otherwise similar to calciferol, it might well account for the differences brought out in the differential test.

But continuing this speculation one would expect the potent fish oils to show the same type of absorption spectrum as calciferol and to slowly form adducts with maleic or citraconic anhydride. Bills has pointed out that the absorption spectra are quite different. Are there any data on the formation of adducts?

My own impression is that the potent substances of the fish oils will be found to be quite different from calciferol but perhaps with the common feature of a conjugated system appropriately placed with respect to other functions. Such seems to have been the idea of Rosenheim and King, also, in one of their speculative articles which appeared a few years ago. This implies a lack of specificity and, although specificity seems to be the rule in nature, the sex hormones as well as the auxines illustrate beautifully that specificity need not be absolute. Of course the physiology of the hormones and vitamins is so obscure that any argument of specificity or non-specificity may be premature. Indeed, it is probable that through a study of various compounds with vitamin D potency a beginning on the physiology may be made.

Dr. Bills: In the narrowest sense, a compound such as calciferol, in which the sterol ring is broken, is not a sterol. But I should prefer to still regard calciferol as a sterol, in the loose sense that we associate phthalic acid with naphthalene, or in the sense that we regard chaulmoogric acid as a fatty acid in spite of its ring structure. It is all a question of how far we should go in a matter of definition.

In answer to Strain's specific question, I will say that little has been done in the formation of adducts from fish oil vitamin D. I recall that there have been one or two attempts, but these did not lead to anything of importance.

Dr. Moyer: Aside from the nutritional aspects of the problem, it may be well to emphasize that the problems of biology are frequently problems concerning heterogeneous systems. It would therefore be of interest to investigate the behavior of calciferol at an interface and compare it with cholesterol or ergosterol. Frequently the properties of molecules at interfaces are different from what one might expect by consideration of their chemical structure. For instance, particles of cholesterol and ergosterol, although secondary alcohols, behave as though amphoteric and exhibit an isoelectric point near pH 3.1 when suspended in buffers. This is probably due to adsorption of ions from the solution but, if so, it is an unusual type of adsorption for other adsorbing surfaces such as cellulose or oil drops do not reverse their sign of charge but remain negative at all pH values in simple salt solutions.

Recent studies, with highly purified crystalline material which had arrived at a steady state with its buffer medium, show that the electrophoretic mobility-pH curves for ergosterol and cholesterol are identical, within the limits of error. It would be of interest to investigate the surface properties of cholestene, cholestane, coprosterol, and dihydrocholesterol in this way, to see if the double bond or the hydroxyl group could be identified as responsible for this behavior. Irradiation of suspended ergosterol crystals or irradiation of films of ergosterol on surfaces might produce an altered surface which could be correlated with changes in charge density. Such investigations carried on with pure materials may lead to a further understanding of the part played by sterols in biological processes.

Dr. Strain: This seems to me to be a rather difficult problem. The sterols form molecular compounds so easily that it is difficult to tell just what one is measuring.

Dr. Meyer: I wonder whether slight structural changes of steric nature would not produce great differences in the physiological behavior of sterols, accounting not only for activation by irradiation, but also by other means. It interested me also to hear Bills' comments on the stability of "ergosterol" in the presence of cholesterol. We found cholesterol completely stable towards oxygen in the presence of heavy metals. A slow catalytic oxidation of cholesterol was only obtained with chlorophyll on irradiation with visible light. On the other hand, it is well known that in tissue extracts, cholesterol is very readily oxidized during saponification, so that it cannot be obtained in crystalline form.

Dr. Brackett: Since calciferol shows an absorption maximum in the region around 265 m μ and relatively less absorption at wavelengths longer than 280 m μ , and since the suprasterols are formed, directly or indirectly by overirradiation, and presumably by initial absorption in the calciferol band, one would expect a larger concentration of vitamin from irradiation with wavelengths of 280 m μ and longer. Is this actually the case? The work of Reerink and van Wijk and others seemed to support this view. Has later work led to a contrary conclusion? The work of Bunker does not indicate much variation in the over-all efficiency for different wavelengths in the general region of ergosterol absorption. However, the results obtained *in vivo* might well be different from those obtained in various solvents.

Dr. Bills: The wavelengths of light employed in irradiation *in vitro* do have an influence on the composition of the product. However, their influence on the amount of vitamin D produced is less than on the relative amounts of other products, such as lumisterol and tachysterol. It is more than likely that the picture is different for sterols irradiated *in vivo*, as in the living skin. There the presence of pigments, filters, stabilizers, and substances with which sterols go into combination might well be factors of importance, but all this is a problem difficult to attack experimentally.

PHOTOSENSITIZED OXIDATION OF ETHYLENIC DOUBLE BONDS

KARL MEYER

The oxidation of substances under the influence of visible light in the presence of fluorescent dyestuffs has two main points of interest: first, a biological, connected with the problem of photodynamic action and the assimilation of CO_2 in the green plant, and second, a chemical and physico-chemical, connected with the problem of autoxidation and chemical reaction mechanism in general. Tappeiner (1) has shown that O_2 is necessary for the photodynamic action. Recently Kautsky (2) has come to the conclusion that O_2 is the only molecule activated under the influence of light and chlorophyll. Though the latter view has been challenged (3, 4, 5), there remains the paramount role played by O_2 in photosynthesis (6).

The following report will confine itself to the study of the photosensitized oxidation of some biologically important substances and its relation to the problem of autoxidation. It seems that a better knowledge of catalytic oxidation in relation to chemical structure and to the catalyst used might be of value for problems of much wider scope.

The experiments reported here were mainly carried out in 1928 and 1929 in the laboratory of Professor Richard Kuhn in Zurich, to whom the author is indebted for much advice. A few experiments have been carried out in collaboration with Dr. John W. Palmer in the author's present laboratory.

EXPERIMENTAL

In most of the experiments the oxygen uptake was measured in a Warburg apparatus at 37° . The light source was a 120 watt single wire filament lamp suspended on movable clamps in the water bath 7 cm. from the bottom of the conical vessels. Aqueous solutions or aqueous-lipoid mixtures were used. Some experiments performed in the author's present laboratory were made with an experimental set-up of a different form which seems to be useful in work of this type. The water bath (Figure 1) has a protruding section (a) at the top with a pane of glass (b) forming the bottom of the extension, as used by Emerson. A removable metal box (c), which fits in under the extension, contains light bulbs (d), one under each manometer, and a cooling fan (e). Above the bulbs are two metal sheets (g), which slide over each other forming slits. The width of the openings made by the slits is adjusted by two screws (h), one at each end of the box. At the top of the box are two slots for inserting glass plates and filters (k). (Our thanks are due Mr. Fred Rosebury of the Department of Bio-

logical Chemistry for the construction of the illuminating apparatus.)

The first experiments were concerned with the oxidation of pyruvic acid (7). This substance was found to be stable toward oxygen in acid

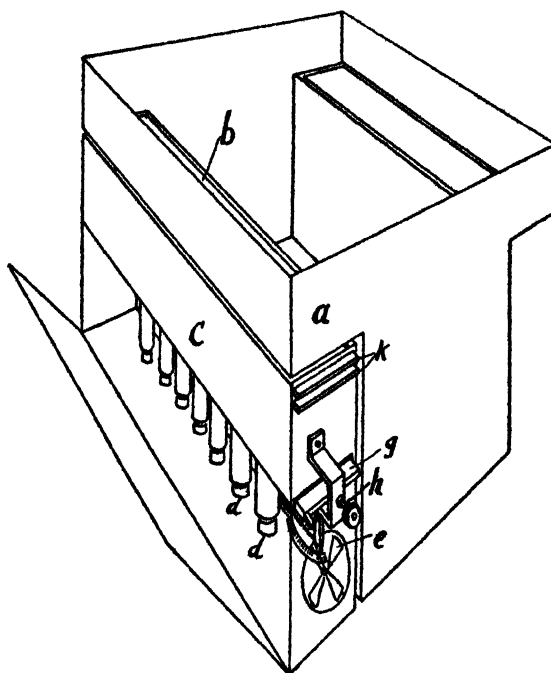


Fig. 1. Apparatus for irradiation of Warburg vessels.

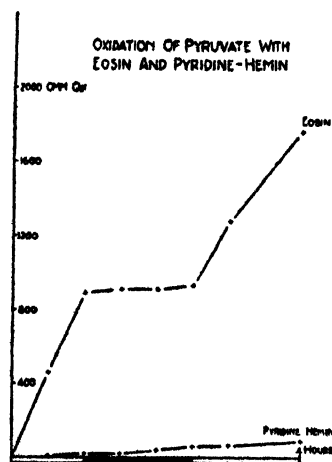


Fig. 2. Oxidation of pyruvic acid with eosin and pyridine-hemin. Each vessel contained 0.3 cc. 0.88 N pyruvic acid + 0.2 cc. 5 N NaOH + 0.9 cc. water + 0.1 cc. addition. Additions: I. 0.1% eosin in N/100 NaOH. II. M/650 pyridine-hemin in 5% aqueous pyridine.

and neutral solutions in the presence or absence of iron. If, however, alkali was added up to a concentration of about 0.8 N, pyruvic acid took up O_2 spontaneously. With pyridine-hemin this oxidation could be greatly accelerated. Light had no influence on the spontaneous oxidation nor on the oxidation with hemin. In the presence of eosin and light the rate of oxidation was many times greater than with hemin (Fig. 2). The oxidation product was oxalic acid, isolated in amounts equivalent to 65-75% of the O_2 up-take. The O_2 uptake was proportional over a wide range to the logarithm of the eosin concentration (Figs. 3 and 4).

Isochlorophylline was found to exert a photosensitizing influence similar to, though smaller than, that with eosin. Rhodamine B was only slightly active. HCN inhibited the spontaneous

oxidation and the catalytic oxidation by hemin, but had no influence on the photosensitized reaction.

Sorbic acid, which has two conjugated double bonds and is oxidized by hemin, was oxidized at neutral reaction with eosin, but much more slowly than pyruvic acid (8). Some natural polyenes (bixin, lycopene) were hardly oxidized at all, apparently because of the conjugation of their double bonds (9).

In further experiments the oxidation of olefinic acids in water-oil two-phase systems was investigated. In a previous study (8) the auto-oxidation of oleic, linoleic, and linolic acids was found to be catalyzed by hemin. The oxidation products were of complex nature, since CO_2 was evolved. Two acids with terminal double bonds, decenic and undecenic, were completely refractory. In the presence of natural chlorophyll¹ dissolved either directly in the substances or in paraffin oil or cyclohexanol, a very rapid O_2 uptake took place upon irradiation (Fig. 5). The

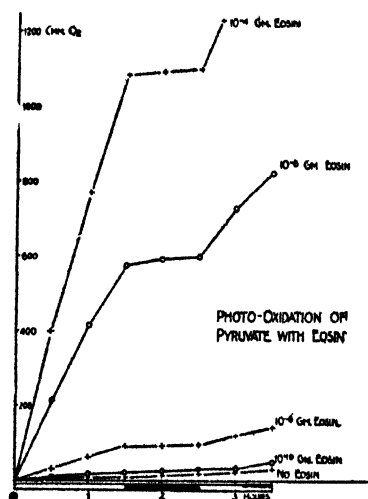


Fig. 3. Photo-oxidation of pyruvic acid with varying amounts of eosin. Each vessel contained 0.3 cc. 0.88 N pyruvic acid + 0.9 cc. water + 0.2 cc. 5 N NaOH + 0.1 cc. N/100 NaOH containing eosin in amount indicated.

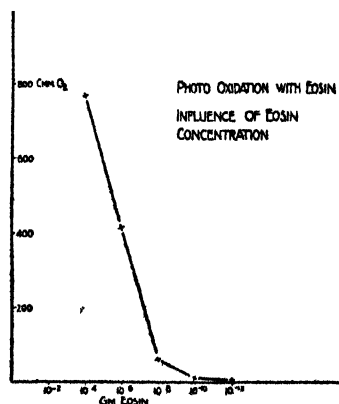


Fig. 4. Photo-oxidation of pyruvic acid, based on data of Fig. 3 at 1 hour.

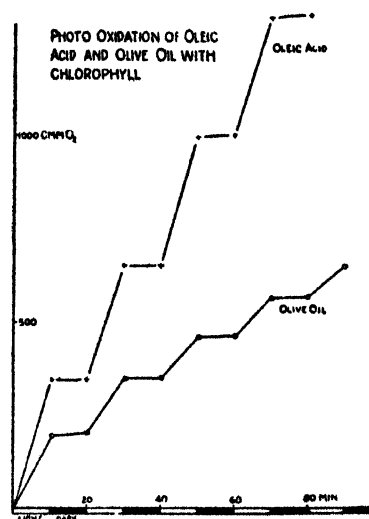


Fig. 5. Photo-oxidation of oleic acid and olive oil with chlorophyll. In each vessel 0.8 cc. M/15 phosphate (pH 7.0) + 0.5 cc. water + 0.1 cc. substrate + 0.1 cc. 0.1% chlorophyll in paraffin oil.

reaction did not yield CO_2 , H_2O_2 , nor organic peroxides. In recent experiments without water the reaction was studied further. It was found that the oxidation of oleic acid slows down after one atom of oxygen has been absorbed. On titrating with alcoholic KOH and simultaneously estimating the iodine number according to the method of Dam (10), it was found that the double bond remained almost unchanged, while

¹ The chlorophyll used was a very pure specimen containing both components A and B in the proportion occurring in the plant. We owe Dr. Stoll of Basle thanks for this specimen.

the equivalent weight had increased to correspond to the oxygen taken up. It is believed that any O_2 uptake beyond 0.5 mol and any change in double bond are due to the simultaneous "dark reaction."

Although the O_2 uptake of the unirradiated control remains small during the short time of the experiments, after irradiation there is a considerable O_2 uptake in the dark, especially during the first minute after irradiation has stopped. This photochemical after-effect is not due to a lag in the O_2 diffusion or to other mechanical factors. As the dark period is prolonged, the O_2 uptake drops to a constant "dark" rate. On addition of hydroquinone in cyclohexanol the dark reaction could be considerably decreased. The light reaction was also decreased, but to a lesser degree. (Fig. 6).

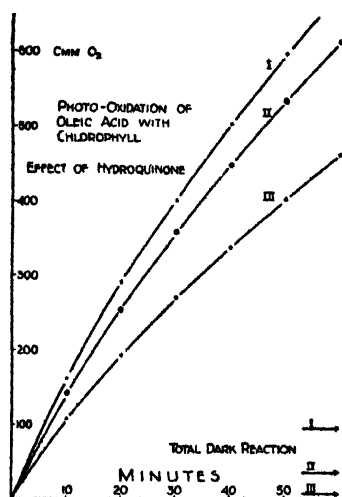


Fig. 6. Photo-oxidation of oleic acid with chlorophyll. Effect of hydroquinone. Each vessel contained 1.2 cc. oleic acid + 0.2 cc. 0.1% chlorophyll in cyclohexanol + 0.1 cc. addition. Additions: I. 0.1 cc. cyclohexanol. II. 0.1 cc. 0.002 M hydroquinone in cyclohexanol. III. 0.1 cc. 0.05 M hydroquinone in cyclohexanol. During the same time the controls, oleic acid + cyclohexanol acid, oleic acid + hydroquinone in cyclohexanol, took up 35.7 and 29.4 cmh. O_2 respectively.

The vessels were irradiated for 10 minutes, after which the manometers were read. A second reading was made after 5 minutes in the dark, and a third after a further 10 minutes in the dark. For the evaluation of the experiments, the O_2 uptake of the 10 minute dark period was taken as giving the true "dark" rate; the dark reaction was assumed to be continuous, and the O_2 uptake during the light period and the 5 minute dark period was corrected for this. The difference between the observed O_2 uptake during the 5 minute dark period and the calculated dark reaction for that time was taken as part of the

true light reaction. This photochemical after-effect is quite pronounced in the viscous solutions, and is probably due to the long life-time of the activated molecules. Kautsky and co-workers have estimated the life span of activated dye molecules from luminescence measurements as 10^{-2} seconds (2).

The estimations of the equivalent weight and the iodine number were made on samples shaken with air in larger flasks. The oxygen uptake was followed gravimetrically. Samples were taken out before the reaction started and at the end. As a control a similar mixture was shaken simultaneously which contained, instead of the chlorophyll solution in cyclohexanol, this solvent only.

In experiments on four substances of terpene nature (citronellal, linalool, pulegone, and terpineol) which showed with or without hemin a negligible oxidation only, a marked photosensitized oxidation was found with chlorophyll (9). Regardless of whether one or two double bonds were present, each substance took up slightly over one mol of oxygen. The decolorization of $KMnO_4$ and of Br_2 when O_2 uptake had ceased indicated that the double bonds were still present. The aldehyde group of citronellal was not attacked.

In experiments on ergosterol (11) the autoxidation, the photochemical oxidation without sensitizer, and the photosensitized oxidation with eosin and chlorophyll were studied. In a diphasic system with aqueous buffer and a lipid solvent, ergosterol showed an autoxidation, greatly accelerated by iron and inhibited by cyanide. More than three mols of oxygen were taken up. With eosin, Windaus and Bruncken (12) had found that, in light, ergosterol absorbs one mol of oxygen, forming an ergosterol peroxide, which was isolated. In the absence of oxygen, eosin acted in stoichiometric proportion as a hydrogen acceptor, forming a dehydroergosterol. In our experiments with eosin in a diphasic system, the oxygen uptake was slightly in excess of one mol. With chlorophyll, the oxygen absorption slowed down after one equivalent was taken up (Fig. 7.)

In these experiments it was observed that the optical rotation of the ergosterol decreased. Such preparations, made in the presence of olive oil or oleic acid, were found to have antirachitic (curative) activity. The antirachitic assays were carried out by Dr. Tschopp of Basle, who used 10 rats for each dilution. The results were checked by X-ray photography. With preparations irradiated in absence of oxygen no healing was observed; neither was activity found in samples irradiated in the absence of chlorophyll, or containing chlorophyll but not irradiated. Still better results were obtained by working in a pure lipid (monophasic) medium. The results are summarized in Table I.

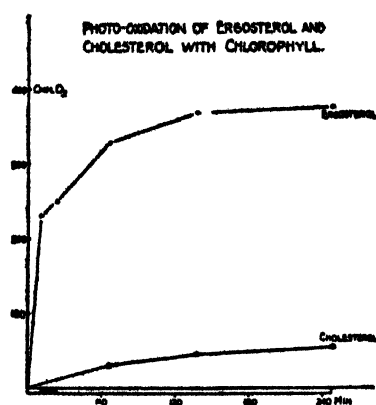


Fig. 7. Photo-oxidation of ergosterol and cholesterol in cyclohexanol with chlorophyll. In each vessel 0.8 cc. $M/15 Na_2HPO_4$ + 0.1 cc. 0.1% chlorophyll in paraffin oil + 0.3 cc. water + 0.1 cc. 3% cholesterol or ergosterol in cyclohexanol.

In similar though not identical experiments, Dr. T. F. Zucker of the Department of Pathology, using the present day standard technique, first did not obtain healing of rachitic rats with our preparations. Later experiments, however, seem to give positive results. It will be desirable to obtain more data on this problem. There is probably a formation and simultaneous destruction of an antirachitic substance. That vitamin D, obtained from ergosterol by irradiation by mercury arc, is destroyed by "autoxidation" and by iron catalysis in the dark at at least the same rate at which ergosterol is oxidized, has been shown by Kuhn and Meyer (8). We find in comparing earlier experiments with oleic acid and chlorophyll with recent experiments on new samples of oleic acid (some had been vacuum-distilled) that the latter show a greater dark reaction and a diminished light re-

TABLE I.

Optical Rotation and Antirachitic Properties of Ergosterol Oxidized in Presence of Chlorophyll

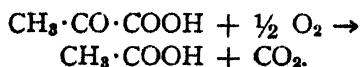
Ergosterol vol. and conc.	Chlorophyll vol. and conc.	Water cc.	Irradiation time hours	Gas	Observed angle	Final ergo- sterol conc.	Tube length cm.	Antirach- itic effect*
5 cc. 3%	0.4 cc. 0.1%	0.6	24	5% CO_2 in N_2	-0.705°	0.484%	2	
"	0	"	"	"	-0.705°	0.485%	2	
"	0.4 cc. 0.1%	"	"	air	-0.005°	0.484%	2	
5 cc. 3%	0.4 cc. 0.1%	0.6	4.5	air	-0.04°	0.484%	1	
"	0	"	"	"	-0.45°	"	"	
"	0.4 cc. 0.1%	"	0	"	-0.49°	"	"	
15 cc. 3%	1.2 cc. 0.1%	1.8	3.0	air	-0.21°	0.60%	1	
"	0	"	"	"	-0.46°	"	"	
"	1.2 cc. 0.1%	"	24	"	-0.025°	"	"	
"	0	"	"	"	-0.32°	"	"	
15 cc. 3%	1.2 cc. 0.1%	1.8	5	air	0.0	0.60%	1	Curative dose 0.1 mg.
"	"	"	0	"				No effect
9 cc. 5%	7.2 cc. 0.1%	1.8	2	air	-0.32°	0.50%	1	
"	"	0	"	"	-0.196°	"	"	
"	"	0	2.5	"	$+0.024^\circ$	"	"	Curative dose 0.1 mg.
"	"	1.8	"	"				Curative dose 0.1 mg.

* Biological activities kindly determined by Dr. Tschopp of "Ciba", Basle. Groups of 10 rats were tested at each level. McCollum diet 3148 was employed. The experimental material was administered orally in sesame oil for three weeks, and the effect was observed roentgenologically. Controls were fed the same material unirradiated, and irradiated but without chlorophyll. Both were without effect. All the animals were protected from light.

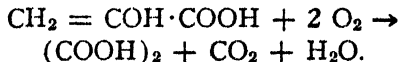
action. In collaboration with Dr. Zucker we will further study this problem and try to suppress the dark reaction, adding a substrate more readily photo-oxidized than oleic acid.

DISCUSSION

The substances which have been found to undergo a photosensitized oxidation with fluorescent dyes are almost all autoxidizable. Table II gives examples which have been studied in greater detail. Most of them possess one or more ethylenic double bonds. The aldehyde group, although autoxidizable (in the presence of heavy metals), is stable toward photosensitized oxidation (formaldehyde, lactaldehyde, glucose, benzaldehyde). Ketones are likewise not photo-oxidized as such. Thus pyruvic acid is oxidized in the keto form in acid solution by H_2O_2 (19), permanganate, etc., to CO_2 and acetic acid:



The keto form is not catalytically oxidized by heavy metals (iron) or Fe-complexes (hemin). In alkaline solution, however, pyruvic acid is catalytically oxidized in absence of light and is likewise photo-oxidized by eosin according to the equation



We have been able to isolate oxalic acid equivalent to 65-75% of the observed oxygen uptake in autoxidation experiments, on oxidation with pyridine-hemin, and with eosin. The speed of the reaction with eosin is about 80 times greater than with hemin.

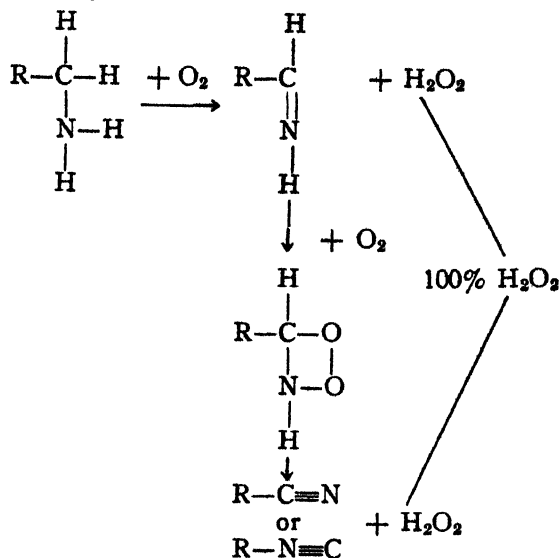
Henri and Fromageot furnished evidence of the enolization of pyruvic acid in alkaline solutions (20). The isolation of oxalic acid as the oxidation product in our experiments proves that it is the enol form which is oxidized. The recent discovery of the phosphorylated derivative of pyruvic acid enol (21) places this compound and its reactions in a dominant role in intermediary sugar metabolism. It will be interesting to study its oxidation *in vitro*.

Among the exceptions to the rule that photo-oxidizable substances are autoxidizable are decenic and undecenic acids, which possess a terminal double bond. An explanation of their lack of autoxidation is difficult, but the fact is in accord with the observation of Böseken (22) that undecenic acid is oxidized by peracetic acid at $1/14$ the rate of oleic acid. This author states that all terminal double bonds are very stable toward such oxidation. In this connection it might be mentioned that cinnamic ester, which was found to be completely resistant to hemin oxidation (8) and

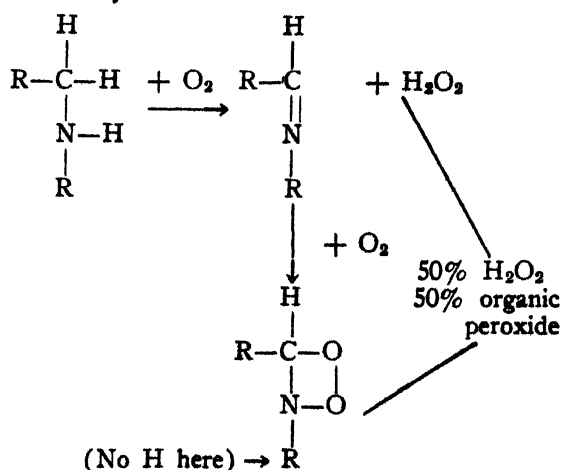
even acted as an inhibitor, was also found by Böseken to be completely resistant to per-acid oxidation.

The amines studied by Gaffron (15) are not unsaturated in the ordinary sense, but nevertheless undergo autoxidation, at least in acetone, and photo-oxidation. However, they may become unsaturated by the formation of imines. The following formulae illustrate a possible mechanism:

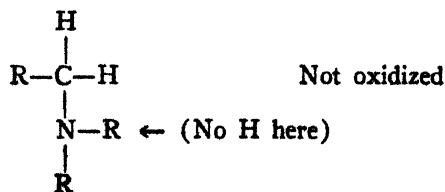
Primary amines:



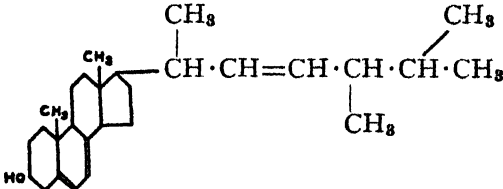

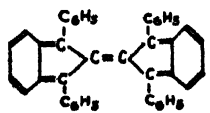
Secondary amines:



Tertiary amines:



TABLE

AUTHOR	SUBSTANCE	STRUCTURE
Harris (13) Gaffron (14)	Tyrosine	$\text{HO} \text{---} \text{C}_6\text{H}_4 \text{---} \text{CH}_2 \text{---} \text{CH} \text{---} \text{NH}_2$ COOH
Gaffron (14)	Uric Acid	$ \begin{array}{c} \text{HN} \text{---} \text{C} \text{: O} \\ \quad \\ \text{O} \text{: C} \quad \text{C} \text{---} \text{NH} \\ \quad \quad > \text{C} \text{: O} \\ \text{HN} \text{---} \text{C} \text{---} \text{NH} \end{array} $
Gaffron (15)	Diisobutylamine Isoamylamine Ethylamine	$ \begin{array}{l} \left[\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3 \end{array} > \text{CH} \text{---} \text{CH}_2 \right]_2 \text{NH} \\ \begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3 \end{array} > \text{CH} \text{---} \text{CH}_2 \text{---} \text{CH}_2 \text{---} \text{NH}_2 \\ \text{CH}_3 \text{---} \text{CH}_2 \text{---} \text{NH}_2 \end{array} $
Gaffron (16)	Allylthiourea	$ \begin{array}{c} \text{NH}_2 \text{---} \text{C} \text{---} \text{NH} \text{---} \text{CH}_2 \text{---} \text{CH} \text{: CH}_2 \\ \\ \text{S} \end{array} $
Windaus and Bruncken (12) Meyer (11)	Ergosterol	
Noack (17)	Benzidine	
Moureu, Dufraisse, et al. (18) Gaffron (3)	Rubrene	
Meyer (7)	Pyruvic Acid	$\text{CH}_2 \text{=C(OH) \cdot COOH}$
	Sorbic Acid	$\text{CH}_3 \text{---} \text{CH} \text{=CH} \text{---} \text{CH} \text{=CH} \text{---} \text{COOH}$
Meyer (9)	Oleic Acid	$\text{CH}_3 \text{---} (\text{CH}_2)_7 \text{---} \text{CH} \text{=CH} \text{---} (\text{CH}_2)_7 \text{---} \text{COOH}$
	Undecenic Acid	$\text{CH}_2 \text{=CH} \text{---} (\text{CH}_2)_8 \text{---} \text{COOH}$
	Citronellal	$\text{CH}_2 \text{=C(CH}_3\text{) \cdot (CH}_2\text{)}_8 \text{---} \text{C(CH}_3\text{)H} \text{---} \text{CH}_2 \text{---} \text{CH} \text{: O}$
	Linalool	$ \begin{array}{c} \text{H}_3\text{C} \\ > \text{C} \text{=CH} \text{---} \text{CH}_2 \text{---} \text{CH}_2 \text{---} \text{C(CH}_3\text{)(OH) \cdot CH} \text{=CH}_2 \\ \text{H}_3\text{C} \end{array} $
	Terpineol	Mixture of four isomers with one double bond in ring or in side chain.
	Pulegone	$ \begin{array}{c} \text{CH}_3 \text{---} \text{HC} < \begin{array}{c} \text{CH}_2 \text{---} \text{C} \text{=O} \\ \text{CH}_2 \text{---} \text{CH}_2 \end{array} > \text{C} \text{=C} < \begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \end{array} $
Meyer (11)	Ergosterol	See above

II.

SENSITIZER	OXIDATION PRODUCT	REMARKS
Hematoporphyrin	$+ 4\frac{1}{2} \text{ mol O}_2 \rightarrow \frac{1}{8} \text{ mol CO}_2$	Autoxidizes
Rose Bengal	$+ 1 \text{ mol O}_2$	Autoxidizes (32)
Ethyl Chlorophyllide	\rightarrow Peroxide	Autoxidizes in acetone
" "	\rightarrow Peroxide + Isonitrile	" " "
" "	\rightarrow Peroxide	" " "
Ethyl Chlorophyllide or Hematoporphyrin	$+ 1\frac{1}{2} \text{ mol O}_2 \rightarrow \frac{1}{2} \text{ mol SO}_2$ (complex reaction)	Autoxidizes
Eosin	$+ 1 \text{ mol O}_2 \rightarrow$ Ergosterol Peroxide	Autoxidizes
Eosin or Chlorophyll	\rightarrow Benzidine blue \rightarrow Diphenolquinonediimine	Autoxidizes
Rubrene	\rightarrow Rubrene Peroxide	
Ethyl Chlorophyllide		
Eosin or Isochlorophylline	$+ 2 \text{ mol O}_2 \rightarrow$ Oxalic Acid	Autoxidizes. Only enol form is oxidized
Eosin		Autoxidizes slowly
Chlorophyll	$+ \frac{1}{2} \text{ mol O}_2 \rightarrow$ Hydroxyoleic Acid (?)	Autoxidizes
Eosin or Chlorophyll		Does not autoxidize
Chlorophyll	$+ \text{ more than } 1 \text{ mol O}_2$	Autoxidizes slowly
"	$+ \text{ " " " " " " }$	" "
"	$+ \text{ " " " " " " }$	" "
"	$+ \text{ " " " " " " }$	" "
Chlorophyll	$+ \frac{1}{2} \text{ mol O}_2 \rightarrow$ No Peroxide	Autoxidizes

A similar reaction mechanism has been demonstrated by Goldschmidt and Beuschel (23) for the oxidation of aliphatic amines by permanganate. The foregoing scheme explains the formation of isonitrile from primary amines as found by Gaffron (15). It also shows why tertiary amines cannot react (15, 23). With the primary amines the peroxide should be all H_2O_2 , and with the secondary amines half should be H_2O_2 and half organic peroxide. In both cases Gaffron found 90% of the total O_2 uptake as peroxide by the liberation of the O_2 with MnO_2 . Naturally this method will not distinguish between H_2O_2 and organic peroxides.

The yield of peroxide in Gaffron's experiments depended upon the solvent used. Thus with acetone he found 90% peroxide, while with water or alcohol only 40-50% was found. Acetone seems to have a stabilizing influence on peroxides. Thus Jorissen and van der Beek (24) were able to isolate from autoxidizing benzaldehyde, dissolved in acetone, perbenzoic acid equivalent to about 60% of the O_2 uptake.

The part of peroxides in autoxidation has been very frequently discussed. In Bayer and Villiger's formulation of benzaldehyde autoxidation (25), perbenzoic acid appears as the intermediary product:



Subsequent investigators (26) found a number of oxidations induced by autoxidizing benzaldehyde which could not be effected by perbenzoic acid. In a previous paper (27) we have shown that perbenzoic acid immediately decomposed into O_2 and benzoic acid under our experimental conditions, whether benzaldehyde was present or absent.

As many investigators have postulated (26) an unstable moloxide of a higher energy content, to which a definite formula cannot be assigned, should be considered as an intermediary in many catalytic oxidations; the moloxide may, under certain conditions, be stabilized to a peroxide, which has been isolated in many instances, in autoxidation as well as in photosensitized oxidation.

It can be seen from Table II that H_2O_2 or an organic peroxide was actually isolated or otherwise demonstrated only with eosin, ethyl chlorophyllide, etc., but not with natural chlorophyll. However in recent experiments on the photo-oxidation of diethylamine in nonaqueous solution with natural chlorophyll peroxide was formed in a concentration of about 50% of the O_2 uptake, using Gaffron's method of decomposing the peroxide with MnO_2 . Differences in reaction products with different catalysts are numerous and widely utilized in thermal reactions industrially.

With eosin, ergosterol takes up one mol of oxygen; with chlorophyll, only 0.5 mol. Oleic acid with chlorophyll also takes up 0.5 mol of oxygen, forming a hydroxyoleic acid. The hydroxyl group does not seem to be at the double bond, since the compound formed then would be an enol and would very likely be oxidized further, or isomerize to the ketone. No evidence of a ketone group was found. The product is probably formed according to Schmidt's rule (28) that the third carbon atom from the double bond on the opposite side from the carboxyl group will be activated. It seems interesting to point out here that ricinoleic acid, a common constituent of plant oils, has this structure.

The difference in reaction products with eosin and chlorophyll points to a different reaction mechanism. Eosin apparently will act easily under irradiation as a hydrogen acceptor. Thus Windaus and Borgeaud (28) obtained dehydroergosterol from ergosterol and eosin in nitrogen. In our own experience eosin can also dehydrogenate diethylamine and oleic acid. Thus the photo-oxidation with eosin in some instances may be explained as a dehydrogenation, with subsequent O_2 addition on the dehydro-substrate and reoxidation of the reduced eosin. That a reduced dye is photo-oxidized has been shown by Kautsky in the case of leuco-malachite green (30). However, substances which under the influence of other catalysts are easily dehydrogenated (as succinic acid and hexose-diphosphate) were found to be completely resistant toward both eosin and chlorophyll.

In contrast to eosin, chlorophyll does not seem to react as a hydrogen acceptor with a substrate (oleic acid or diethylamine) in absence of oxygen. A solution of chlorophyll in olive oil protected from oxygen can be exposed to light for weeks without losing its color or fluorescence.

J. Franck (5) has proposed a reaction mechanism for the role of chlorophyll and O_2 in the assimilation of CO_2 . In this scheme the ordinary chlorophyll is represented as HH-chlorophyll, which reacts with O_2 and H_2O to form H-chlorophyll (mono-dehydrochlorophyll) and HO-chlorophyll. If the same primary reactions take place in the photo-oxidation with chlorophyll, HO-chlorophyll would exchange its hydroxyl for one labile hydrogen atom of the substrate. The difference between eosin and chlorophyll thus seems to be that the former accepts hydrogen in a one-step reaction and the latter in a two-step reaction (31). The two very similar-appearing photosensitized oxidations would represent different reactions mechanisms. Perhaps a better understanding of oxidations in general, thermal and photosensitized, could be reached if they were not represented as following *one* scheme.

SUMMARY

In general, substances are oxidized in a photosensitized reaction if they undergo autooxidation under similar conditions. They are either unsaturated or exist partly in an unsaturated form, as pyruvic acid in the enol form or primary and secondary amines as imines. The aldehyde and ketone groups as such are stable in this reaction.

Certain investigators have isolated, or otherwise demonstrated, peroxides as oxidation products, often in nearly quantitative yield. In other cases peroxides may be formed in decreased yields, or even not at all. In some instances the solvent influences the yield of peroxide (amines). Pyruvic acid in alkaline solution yields oxalic acid as a photo-oxidation product, while H_2O_2 and other per-acids in acid solution oxidize it to acetic acid. In the oxidation of oleic acid, some terpenes, and ergosterol in the presence of chlorophyll, which is here a much more active catalyst than eosin, no peroxide could be demonstrated. With chlorophyll, only 0.5 mol of oxygen is taken up by ergosterol and oleic acid, the latter forming a hydroxyoleic acid.

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